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Transcriptional Activation by TFIIB Mutants That Are Severely Impaired in Interaction with Promoter DNA and Acidic Activation Domains

SUSANNA CHOU AND KEVIN STRUHL*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

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Biochemical experiments indicate that the general transcription factor IIB (TFIIB) can interact directly with acidic activation domains and that activators can stimulate transcription by increasing recruitment of TFIIB to promoters. For promoters at which recruitment of TFIIB to promoters is limiting in vivo, one would predict that transcriptional activity should be particularly sensitive to TFIIB mutations that decrease the association of TFIIB with promoter DNA and/or with activation domains; i.e., such TFIIB mutations should exacerbate a limiting step that occurs in wild-type cells. Here, we describe mutations on the DNA-binding surface of TFIIB that severely affect both TATA-binding protein (TBP)–TFIIB–TATA complex formation and interaction with the VP16 activation domain in vitro. These TFIIB mutations affect the stability of the TBP-TFIIB-TATA complex in vivo because they are synthetically lethal in combination with TBP mutants impaired for TFIIB binding. Interestingly, these TFIIB derivatives support viability, and they efficiently respond to Gal4-VP16 and natural acidic activators in different promoter contexts. These results suggest that in vivo, recruitment of TFIIB is not generally a limiting step for acidic activators. However, one TFIIB derivative shows reduced transcription of *GAL4*, suggesting that TFIIB may be limiting at a subset of promoters in vivo.

Transcription factor IIB (TFIIB) is an essential component of the RNA polymerase II (Pol II) machinery that acts as a bridge between the TATA-binding protein (TBP) and Pol II (42, 49). The C-terminal domain of TFIIB interacts with the TBP-TATA complex, whereas the N-terminal domain is required for the assembly of TFIIF and Pol II into the preinitiation complex. In the TBP-TFIIB-TATA complex, TFIIB directly contacts TBP as well as DNA sequences immediately upstream and downstream of the distorted TATA element (35, 41). In stepwise assembly reactions in vitro, TFIIB becomes stably associated with the promoter after the binding of TBP to the TATA element but prior to the incorporation of TFIIF, TFIIE, TFIIH, and Pol II. However, TFIIB is present in several Pol II holoenzyme preparations, leading to the possibility that it may be recruited to promoters as part of a large macromolecular complex (30, 32, 55). In addition, TFIIB interacts with certain TBP-associated factors (TAFs) in vitro (16), and these interactions might contribute to recruitment or stable association of TFIIB in the context of an active transcription complex.

Numerous biochemical studies endorse a role for TFIIB in the response to transcriptional activator proteins. TFIIB can directly bind to diverse classes of activation domains, and recruitment of TFIIB to the TBP-TATA complex is enhanced by the acidic activator VP16 and the proline-rich activator CTF1 (29, 37). Mutant VP16 acidic activation domains that fail to activate transcription also do not interact with TFIIB (38). Conversely, TFIIB point mutants that do not interact with the VP16 activation domain support basal but not activator-dependent levels of transcription (46, 48). Aside from their ability to increase TFIIB recruitment, acidic activators can also induce a conformational change in TFIIB that disrupts an intramolecular interaction between the N- and C-terminal domains and stimulates preinitiation complex formation (47). These observations indicate that TFIIB can be a target for activation domains under defined experimental conditions, but they do not address whether TFIIB is a significant target under physiological conditions.

In vivo, several observations are consistent with the idea that TFIIB is an important target for activator proteins, but definitive evidence is lacking. First, there is an excellent correlation between the strength of the activator-TFIIB interaction in vitro and transcriptional activity in vivo (40, 60). However, an equally suggestive correlation has been observed for activator-TBP interactions, and other potential targets have not been examined at this level of resolution. Second, artificial recruitment of TFIIB by fusion to a promoter-bound protein enhances transcription (13, 34), indicating that an activator-TFIIB interaction could, in principle, be sufficient for activation. However, artificial recruitment experiments do not address which components are targets of natural activators, particularly because similar results are observed when TBP (8, 31, 61), TAFs (1, 28), or components of the Pol II holoenzyme (4, 27, 45) are artificially recruited to promoters. Third, in accord with the observation that TFIIB is generally required for transcription of Pol II genes in yeast cells (39), human TBP derivatives that are severely defective for interacting with TFIIB are transcriptionally inert on most promoters in HeLa cells (7, 58). However, these results do not distinguish between activator-specific effects and general effects on Pol II transcription, and they do not address whether the TBP-TFIIB interaction is limiting in wild-type cells. Fourth, mutations in yeast TFIIB can differentially affect genes that are responsive to distinct activator proteins (51), although it is unclear if these effects are activator specific. Moreover, the relationship to TFIIB recruitment to promoters can not be assessed, because the biochemical properties of these TFIIB derivatives have not been described. Fifth, overexpression of an N-terminal fragment of *Drosophila* TFIIB can squelch activation by a glutamine-rich, but not an

* Corresponding author. Fax: (617) 432-2529. E-mail: kevin@hms.harvard.edu.

acidic, activation domain (10, 11). The relevance of this observation to the physiological mechanism of transcriptional activation is unclear.

In apparent contrast to the above-described observations, a severely defective TBP-TFIIB interaction does not preclude transcriptional activation in vivo. In yeast cells, TBP derivatives with mutations on the TFIIB interaction surface cause 50- to 100-fold defects in TBP-TFIIB-TATA complex formation in vitro yet are generally competent for transcriptional activation in vivo (34). In the more extreme case, activation is observed even though the TBP derivative is unable to support cell growth and hence is limiting for the TBP-TFIIB interaction at some promoters. In contrast, there are several examples of activation-defective TBP derivatives that can support cell growth (2, 33, 52, 53). In accord with these observations, a human TBP derivative with a 40-fold defect in TBP-TFIIB-TATA complex formation in vitro is competent for activation in vivo (7). TFIIB interaction mutants of human TBP with more severe defects are generally impaired for transcription in vivo (7, 58), although activation by Sp1 is only modestly affected (58). These results suggest that the TBP-TFIIB interaction, and perhaps TFIIB itself, is not generally limiting for transcriptional activation in vivo.

To more directly address whether recruitment of TFIIB is generally limiting for transcriptional activation in vivo, we analyzed the transcription properties of TFIIB mutants with defined biochemical properties. The X-ray structure of the TBP-TFIIB-TATA complex identifies several contacts between side chain residues of human TFIIB and the sugar-phosphate backbone of the DNA flanking the TATA element (41). Interestingly, protease footprinting (22) and mutational analysis (38) indicate that this region of TFIIB interacts with the VP16 activation domain in vitro. Moreover, two of these DNA-interacting residues are altered in the human TFIIB mutants exhibiting defects in VP16-dependent activation in vitro (46, 48). We therefore characterized the transcriptional properties of yeast TFIIB derivatives with analogous substitutions on the DNA-binding and VP16 interaction surfaces. Our results suggest that TFIIB is not generally limiting for transcriptional activation in vivo but that specific mutations on the DNA-binding surface can exert promoter-specific effects on transcription.

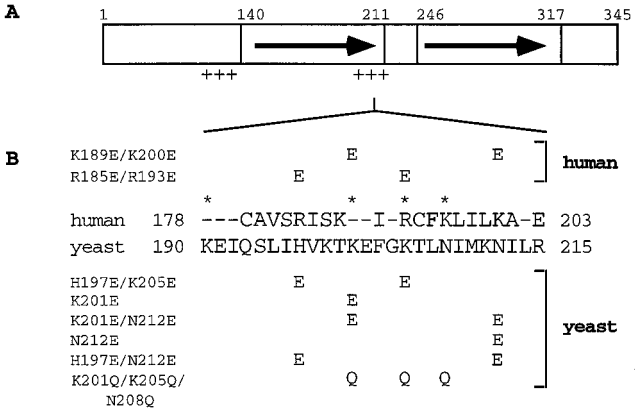

FIG. 1. Yeast and human TFIIB mutants. (A) Diagram of TFIIB structure, with repeated domains (arrows) and basic repeats (+++) in core TFIIB indicated. Amino acid numbers refer to positions in yeast TFIIB. (B) Alignment of the second basic repeat with mutations in human TFIIB (20) and yeast TFIIB (44) at the indicated amino acid positions: E, basic residues mutated to glutamate in human (48) and yeast (this work) TFIIB; Q, yeast TFIIB residues mutated to glutamine in this work; *, residues with side chain contacts to DNA in the TFIIB-TBP-TATA cocrystal structure (41).

MATERIALS AND METHODS

DNAs and yeast strains. TFIIB mutants were constructed in a derivative containing *Eco*RI and *Bsp*HI sites at positions +604 and +625 from the start of translation, respectively, by PCR site-directed mutagenesis. Yeast strains ySH1 (*MAT***a**) and ySH2 (*MAT*α) were derived from KY320 (9) by integrating the TFIIB *Cla*I-*Nco*I fragment deleted from residues −316 to +168 and having the *ADE2* gene inserted at residue +971. The deletion allele was complemented with a *URA3* centromeric plasmid expressing wild-type TFIIB under the control of its natural promoter. The wild-type *his3* promoter and derivatives containing binding sites for the activator Gcn4, Gal4, or Ace1 (24) were introduced into ySH1 by replacement of the chromosomal *his3* locus. Mutant TFIIB derivatives were introduced into ySH1 and its derivatives by plasmid shuffling involving growth on medium containing 5-fluoroorotic acid (6).

Strain ySH12 was derived from BYΔ2 (12) by disruption of the locus encoding TFIIB with *ADE2* as described above. In this strain, the TFIIB and TBP deletion alleles are complemented with a *URA3* centromeric plasmid expressing both wild-type TFIIB and TBP under the control of their normal promoters. For determination of synthetic lethality, *TRP1* centromeric plasmids bearing TFIIB alleles were cotransformed with *LEU2* centromeric plasmids bearing TBP alleles, and the transformants were spotted on plates containing 5-fluoroorotic acid. To construct ySH15, which bears the *GAL4-CAT* integrated reporter, the *Sac*I-*Kpn*I fragment of plasmid BM1974 (17), which contains the *URA3* marker and the *GAL4* promoter fused to the chloramphenicol acetyltransferase (CAT)-coding sequence, was used to replace the *GAL4* locus in ySH1. The Gal4-VP16 activator used for transcriptional assays in vivo was derived from pSB201 (5). A *Bam*HI fragment containing the *ADH1* promoter, the *GAL4* DNA-binding domain fused to the full-length VP16 activation domain (residues 413 to 490), and 350 bp of the *ADH1* termination region was ligated into the *Bam*HI site of a *URA3* centromeric plasmid.

Protein purification. TFIIB derivatives were subcloned into a pET11 plasmid containing the wild-type TFIIB-coding sequence (obtained from Steve Buratowski) by swapping the *Bsp*EI-*Asp*718 fragment within the coding region, and proteins were expressed in *Escherichia coli* BL21(DE3). Cell pellets from a 500-ml culture were sonicated in 4 to 5 ml of lysis buffer (10 mM Tris-acetate [pH 7.9], 1 mM EDTA, 10% glycerol, 500 mM potassium acetate, 1 mM dithiothreitol [DTT], 2 mM phenylmethylsulfonyl fluoride [PMSF]). The clarified lysate was diluted to 100 mM potassium acetate with buffer T (10 mM Tris-acetate [pH 7.9], 1 mM EDTA, 10% glycerol, 2 mM PMSF) and incubated with a 0.5-ml bed volume of S-Sepharose FF (Pharmacia) that was equilibrated with buffer T plus 100 mM potassium acetate (buffer T+0.1) at 4°C with gentle agitation. After three washes with 10 column volumes of buffer T+0.1, the resin was poured into a column and proteins were eluted over a gradient from 100 mM to 1 M potassium acetate. Peak fractions were pooled, aliquoted, and frozen at −70°C. Purified proteins were quantitated by the Bradford assay (Bio-Rad) and were approximately 90% pure as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining. TFIIB appeared as a doublet comprising a 39-kDa full-length species and a 29-kDa proteolytic fragment corresponding to the C-terminal core. Recombinant human TFIIB was expressed from the plasmid pET-6His-hIIB, and the bacterial lysate was purified on a nickel-agarose column (Novagen), as described by manufacturers except that all buffers contained 10% glycerol and contained potassium acetate instead of sodium chloride; the preparation was approximately 80% pure.

Analysis of TBP-TFIIB-TATA complex formation. Binding reaction mixtures (11 μl) contained the following ingredients: 6 μl of buffer T+0.1, 6 μg of bovine serum albumin, 7 mM magnesium acetate, 300 μg of poly(dG-dC), and 0.5 nM ^{32}P-labeled DNA containing the E1B TATA element. For each reaction mixture containing TBP, 7.5 ng of recombinant yeast TBP was added. Binding reaction mixtures were incubated at room temperature for 20 min prior to electrophoresis through 0.5× Tris-borate-EDTA–5% polyacrylamide gels at room temperature.

Interaction with the VP16 activation domain. VP16 wild-type and mutant activation domains were expressed as glutathione *S*-transferase (GST) fusions from plasmids pGVP and pGVPΔ456-F442P (38). Cultures of *E. coli* XA90 transformed with pGEX-2T, pGVP, and pGVPΔ456-F442P were induced with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), and the bacterial lysate was purified on preequilibrated glutathione-Sepharose beads (Pharmacia) according to the manufacturer's instructions; beads containing the coupled GST fusion proteins were stored as a 50% slurry phosphate-buffered saline at −70°C. For each analysis, the beads containing the GST derivatives were adjusted to a concentration of 0.5 mg/ml with preequilibrated glutathione-Sepharose beads. A settled bed volume of 10 μl (5 μg of protein) was washed three times with 100 μl of buffer T+0.1 containing 0.03% Nonidet P-40, 1 mM DTT, and 2 mM PMSF and then was brought to a final volume of 100 μl in this wash buffer. Two hundred nanograms of recombinant yeast or human TFIIB was then added, and the tube was incubated with gentle agitation for 30 min at 4°C. The beads were collected by brief centrifugation and washed five times with 100 μl of wash buffer, and the protein was eluted in 25 μl of 2× SDS-Laemmli protein loading buffer and heated at 70°C for 5 min. Bound TFIIB was detected by SDS-PAGE and

subsequent Western blot analysis with antibodies against human TFIIB (Santa Cruz Biotechnologies) or yeast TFIIB (a gift of Alfred Ponticelli).

Trypsin cleavage of recombinant TFIIB. Recombinant TFIIB derivatives were diluted to a concentration of 40 μg/ml in cleavage buffer (buffer T+0.1 containing 0.5 mM DTT and 10 mM $CaCl_2$). For each reaction, to 1 μl of TFIIB was added 9 μl of cleavage buffer containing 0, 5, or 25 ng of trypsin-TPCK (tolylsulfonyl phenylalanyl chloromethyl ketone) (Worthington). Reaction mixtures were incubated for 5 min at room temperature, and the cleavage products were separated by SDS-PAGE and analyzed by Western blotting with antibodies against yeast TFIIB.

Transcriptional analysis. For all experiments assaying Gcn4-dependent activation, strains were grown in yeast extract-peptone-dextrose (YPD) to an A_{600} of ~0.4, washed, and then induced for 4 h with 10 mM aminotriazole in synthetic minimal (SD) medium lacking histidine. For assay of Gal4-dependent activation and *GAL4* transcription, strains were grown in YP plus 2% galactose and 0.1% glucose. For assay of Ace1-dependent activation, strains were grown in synthetic complete (SC) medium to early log phase and induced with 100 μM $CuSO_4$ for 1 h before harvesting. Gal4-VP16-dependent activation was assayed in strains with or without the Gal4-VP16 expression plasmid that were grown in SD medium lacking uracil and containing 0.6% Casamino Acids. Total RNA (40 μg as quantitated by A_{260}) was hybridized to completion with a 10- to 100-fold excess of the appropriate ^{32}P-labeled oligonucleotides and treated with S1 nuclease as described previously (26). Transcript levels were quantitated with respect to the *DED1* internal control by phosphorimage analysis (Fujix). RNA levels are expressed as molecules per cell, based on the previously determined value of 25 molecules/cell for *DED1* mRNA in YPD or glucose-SC medium (26). The *CUP1*, *DED1*, *GAL1*, *HIS3*, and *HIS4* oligonucleotides have been described previously (9, 26, 52). The oligonucleotide used to assay *GAL4* transcription was GTCGG CAAATATCGCATGCTTGTTCGATAGAAGACAGTAGCTTCATCTTTCA GGAGGCTTGCTTCTCTGAAGAGA. The error for individual mRNA determinations is ±25%.

CAT assays. Protein extracts were prepared and CAT activities were measured as described previously (17). For each extract, 10 μg of protein was assayed.

RESULTS

General approach. It has been suggested that recruitment of TFIIB to promoters may be limiting for transcription and that activators can stimulate transcription by directly interacting with TFIIB and enhancing its recruitment (see the introduction). A prediction of this hypothesis is that for promoters at which TFIIB recruitment is limiting, transcriptional activity should be particularly sensitive to TFIIB mutations that decrease the association of TFIIB with promoter DNA and/or with activation domains; i.e., such TFIIB mutations should exacerbate a limiting step that occurs in wild-type cells. To address whether TFIIB recruitment is limiting under physiological conditions, we generated yeast TFIIB mutants defective for interaction with DNA and/or activation domains and analyzed their transcriptional properties in yeast cells. To obtain such TFIIB derivatives, we mutated residues in yeast TFIIB that are homologous to human TFIIB residues important for interaction with the VP16 activation domain (38, 48) or implicated in DNA backbone contacts by the X-ray structure (41) (Fig. 1). Although the C-terminal domains of yeast and human TFIIBs are only 60% similar in sequence, they are functionally

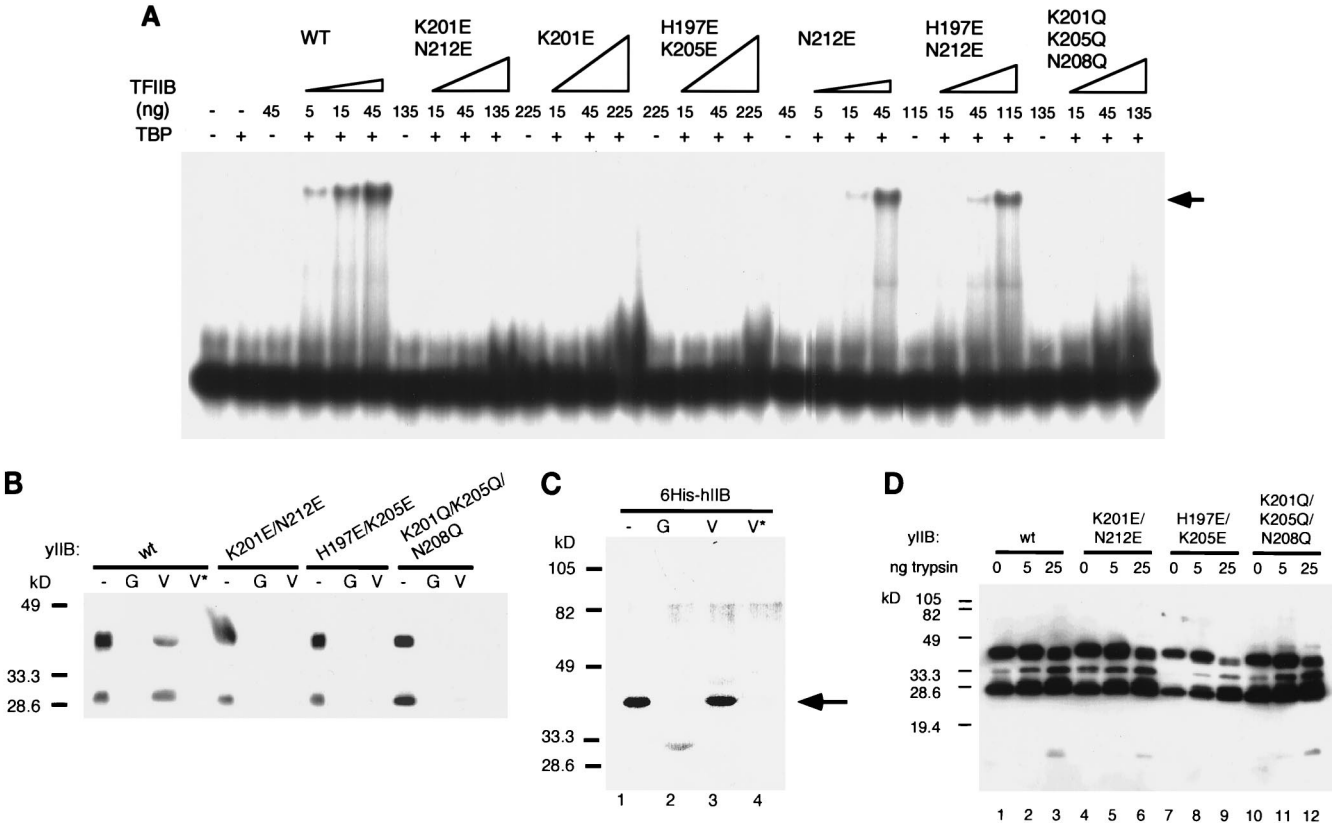

FIG. 2. Analysis of TFIIB mutants in vitro. (A) TFIIB-TBP-DNA complex assembly. The indicated amounts of purified recombinant TFIIB derivatives (WT, wild type) were incubated with 0.3 pmol of histidine-tagged yeast TBP and 5 fmol of TATA element-containing probe. The arrow indicates the position of the TFIIB-TBP-DNA complex. (B) Affinity chromatography assay for yeast TFIIB interaction with VP16 activation domain. Two hundred nanograms of each yeast TFIIB (yIIB) derivative was incubated with a 250-fold excess of GST (G), GST-VP16 (V), or GST-VP16Δ456FP442 (V*) protein coupled to glutathione agarose beads. Forty nanograms of each yeast TFIIB derivative was directly loaded in lanes 1, 4, 7, and 10. The upper band corresponds to full-length 38-kDa TFIIB, and the lower band corresponds to the 29-kDa core TFIIB proteolytic fragment which possesses both DNA- and VP16-binding activities. wt, wild type. (C) Human TFIIB interaction with VP16 activation domain. Purified histidine-tagged human TFIIB (6His-hIIB) was analyzed as described for panel B. The arrow indicates the position of human TFIIB. Other faint bands are due to antibody cross-reactivity with GST fusion proteins. (D) Trypsin cleavage of yeast TFIIB derivatives. Forty nanograms of each TFIIB derivative was incubated with the indicated amounts of trypsin-TPCK for 5 min at room temperature and analyzed by immunoblotting.

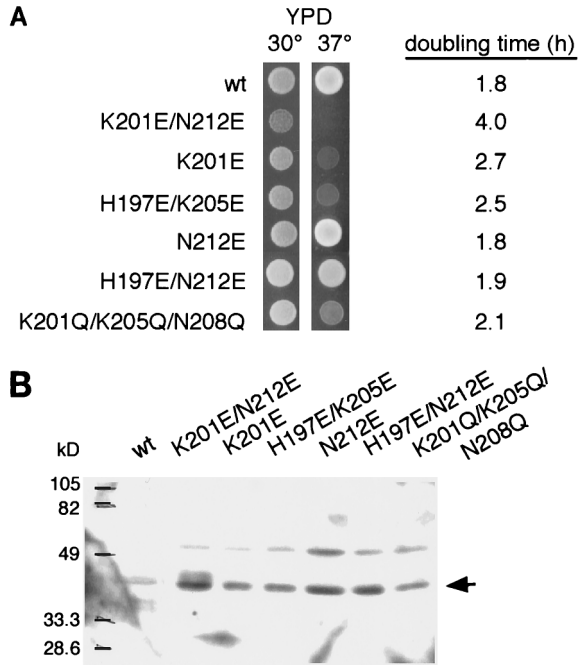

FIG. 3. Phenotypic analysis of TFIIB mutant strains. (A) Cells of wild-type (wt) or TFIIB mutant strains were spotted on YPD plates containing 5-fluoroorotic acid at 30 or 37°C. Doubling times in liquid culture were determined in YPD medium at 30°C. (B) Western blot analysis of strains supported by mutant TFIIB alleles, with the position of TFIIB marked by an arrow.

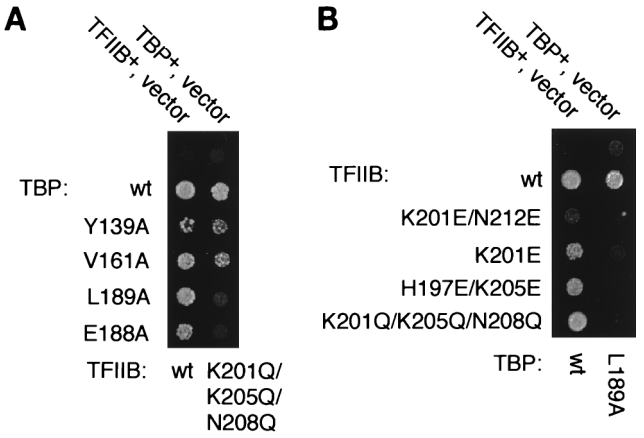

FIG. 4. Synthetic lethality between TBP and TFIIB mutant alleles. DNAs containing the indicated TFIIB and TBP alleles were cotransformed into ySH12 bearing the wild-type (wt) alleles on a single *URA3* centromeric plasmid and then spotted on plates containing 5-fluoroorotic acid.

interchangeable for TBP-TFIIB-TATA complex formation with either yeast or human TBP, and the TBP-TFIIB interface is conserved at the mutational level (34, 56).

Yeast TFIIB mutants that are defective for TBP-TFIIB-DNA complex formation and/or interaction with the VP16 activation domain. Yeast TFIIB mutants were expressed in *E. coli*, purified, and tested for their ability to bind yeast TBP-TATA complexes by electrophoretic mobility shift analysis (Fig. 2A). All mutants containing changes at predicted DNA contact positions (K201, K205, and N208) are compromised greater than 30- to 50-fold for TBP-TFIIB-TATA complex formation. In contrast, derivatives containing mutations at other positions in the general region (N212E and H197E/N212E) are affected only slightly, about threefold. The behavior of the mutant yeast proteins is consistent with the crystal structure of the TBP-TFIIB-TATA complex containing human TFIIB, and the severity of the TBP-TATA binding defect in the K201Q/K205Q/N208Q mutant is similar to that observed for the homologous *Drosophila* TFIIB triple mutant (63). Thus, the interface between TFIIB and the TBP-TATA complex is conserved between yeast, *Drosophila*, and human.

To determine if the affinity of TFIIB for VP16 in vitro is also conserved between yeast and human, yeast TFIIB and the three derivatives with the most marked TBP-TATA binding defects, i.e., the K201E/N212E, H197E/K205E, and K201Q/K205Q/N208Q mutants, were tested for their ability to interact with the activation domain of VP16 (Fig. 2B). In apparent contrast to a previous observation (19), wild-type yeast TFIIB behaves indistinguishably from human TFIIB (Fig. 2C); it binds strongly to the VP16 activation domain but is unable to bind to the transcriptionally inactive F442P mutant. Consistent with the mutants of human TFIIB (48), both of the homologous yeast mutants (K201E/N212E and H197E/K205E) as well as the K201Q/K205Q/N208Q mutant are severely defective for interaction with the VP16 activation domain. Trypsin cleavage of the three mutant proteins reveals no significant differences in kinetics or cleavage products compared to wild-type TFIIB (Fig. 2D), suggesting that the mutant proteins are structurally intact.

TFIIB mutants support cell growth in a manner that is not correlated with TBP-TFIIB-TATA complex formation in vitro. The TFIIB mutants described above were expressed from the natural TFIIB promoter on a single-copy plasmid and tested for their ability to support yeast cell growth (Fig. 3A). All of TFIIB derivatives support viability, although some of them show slow-growth phenotypes at 30°C (the K201E/N212E mutant severely and the K201E and H197E/K205E mutants mildly) and are temperature sensitive. Western blot analysis indicates that TFIIB levels in the mutant strains are comparable to that in a wild-type strain (Fig. 3B), indicating that the growth phenotypes reflect the functional quality of the TFIIB derivatives. Interestingly, the K201Q/K205Q/N208Q derivative, which is severely defective for TBP-TFIIB-TATA complex formation and for interaction with the VP16 activation domain, grows at near-wild-type rates at 30°C and is only moderately temperature sensitive. Thus, the severity of the growth defects does not correlate with impairment in TBP-TFIIB-TATA complex formation.

Synthetic lethal interactions with TBP mutants. The K201Q/K205Q/N208Q derivative of TFIIB efficiently supports cell growth at 30°C despite its marked defect in forming the TBP-TFIIB-TATA complex in vitro. We asked whether this TFIIB mutant is defective in TBP-TFIIB-TATA complex formation in vivo by looking for synthetic lethal interactions with previously described TBP mutants that are defective for specific interactions (33, 34). When the TFIIB derivative is combined with TBP-Y139A (specifically defective for interacting with TFIIA) or TBP-V161A (defective for TATA element binding due to a mutation on the DNA-binding surface), the resulting strains grow quite well at 30°C (Fig. 4A). In contrast, when the TFIIB mutant is combined with TBP derivatives that are defective for interacting with TFIIB (TBP-L189A, which shows a 10-fold defect in TBP-TFIIB-TATA complex formation, and the more severely defective TBP-E188A, which shows a 50-fold defect), the resulting strains grow extremely slowly (L189A mutant) or are inviable (E188A mutant) (Fig. 4A). The synthetic interaction with TBP-L189A is noteworthy, because this

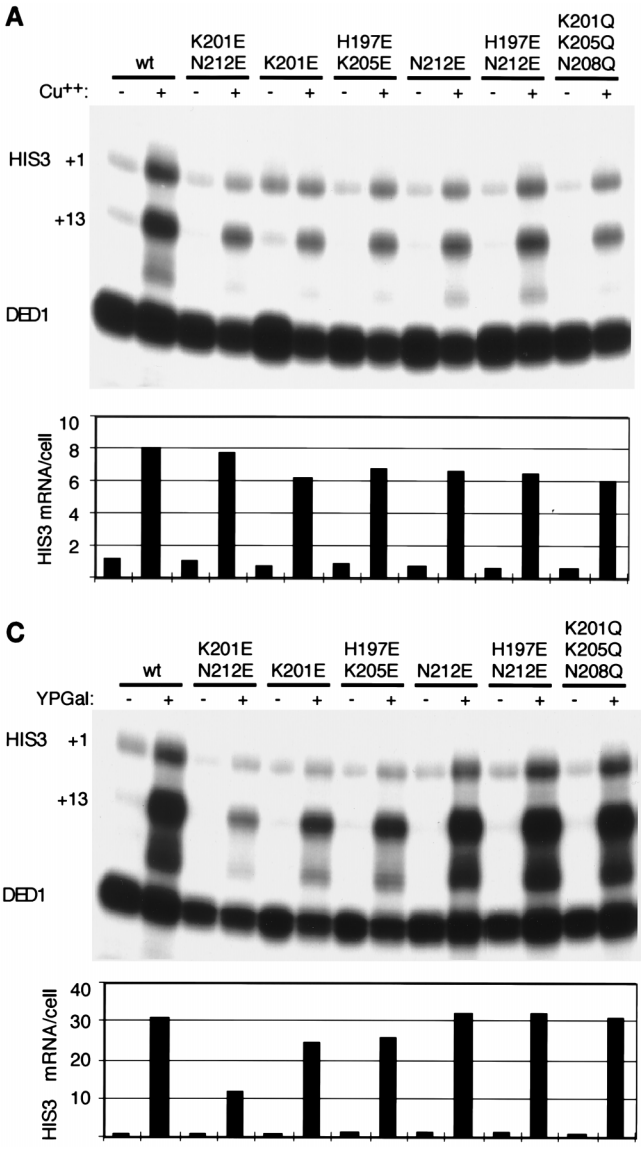

FIG. 5. Activated transcription in the *HIS3* promoter context in TFIIB mutant strains. *HIS3* RNAs were quantitated by phosphorimager analysis and normalized to *DED1* RNA levels, which were previously determined to be 25 molecules/cell (26). (A) Ace1-dependent activation. (B) Gcn4-dependent activation. (C) Gal4-dependent activation. wt, wild type; 3′-AT, aminotriazole.

TBP derivative confers growth properties that are indistinguishable from those of wild-type TBP. These results strongly argue that the K201Q/K205Q/N208Q derivative of TFIIB is impaired for TBP-TFIIB-TATA complex formation in vivo. Synthetic lethality with TBP-L189A is also observed with other TFIIB derivatives containing mutations on the DNA-binding surface (Fig. 4B).

TFIIB mutants do not generally affect transcriptional activation in vivo. We examined the transcriptional response of the TFIIB mutants to various activators and on a variety of promoters. The TFIIB mutants were first assayed on a set of *his3* promoters in which binding sites for the strong activators Ace1, Gal4, and Gcn4 are located upstream of the *his3* TATA region (24). As the promoters are identical with the exception of the activator binding site, any differences in the response of a given TFIIB mutant must be due to the effects of the individual activators rather than promoter context. With the exception of the K201E/N212E derivative, all of the TFIIB mutants exhibit wild-type responses to all three of the activators mentioned above (Fig. 5), as well as to heat shock factor (data not shown). The K201E/N212E derivative has normal responses to Ace1 and Gcn4, but its activity on the Gal4-dependent promoter is reduced to about 50% of wild-type levels.

We also considered the possibility that an effect on transcriptional activation might be observed only in the context of a different promoter architecture. We therefore analyzed these RNA preparations for transcription from natural promoters that are induced by the relevant activators (Fig. 6). In addition, we measured transcription from the wild-type *HIS3* promoter to determine if the natural upstream sequences, which include a poly(dA · dT) stretch that imposes a more open conformation on the promoter region (25), affect activation in the mutant strains. As seen in Fig. 6, activation from the natural promoters is also unaffected in most of the mutant TFIIB backgrounds. Thus, transcriptional activation in vivo is not generally affected by TFIIB mutations that severely impair the interaction with DNA or with the VP16 activation domain. However, in accord with results for the Gal4-dependent *his3* promoter, the activation of the natural *GAL1* promoter is decreased about fourfold in the strain containing K201E/N212E.

Finally, as the biochemical experiments were performed with the VP16 activation domain, we examined the TFIIB derivatives for their ability to support activation by Gal4-VP16 (Fig. 7). When assayed on the *GAL1* promoter, the TFIIB mutants behave indistinguishably from wild-type TFIIB, with the exception of the K201E/N212E mutant, which appears to activate transcription about half as efficiently.

The K201E/N212E mutant affects transcription of the *GAL4* gene. The K201E/N212E mutant might affect Gal4-dependent transcriptional activation either directly, by perturbing the function of the Gal4 activation domain, or indirectly, by modifying the expression of the *GAL4* gene. To determine if such indirect effects are at least partially responsible for the Gal4 activation phenotype of the K201E/N212E mutant, *GAL4* expression was evaluated by measuring RNA levels and by determining CAT activity from a reporter containing the structural gene for this enzyme under the control of the *GAL4* promoter. By both of these assays, *GAL4* expression is reduced

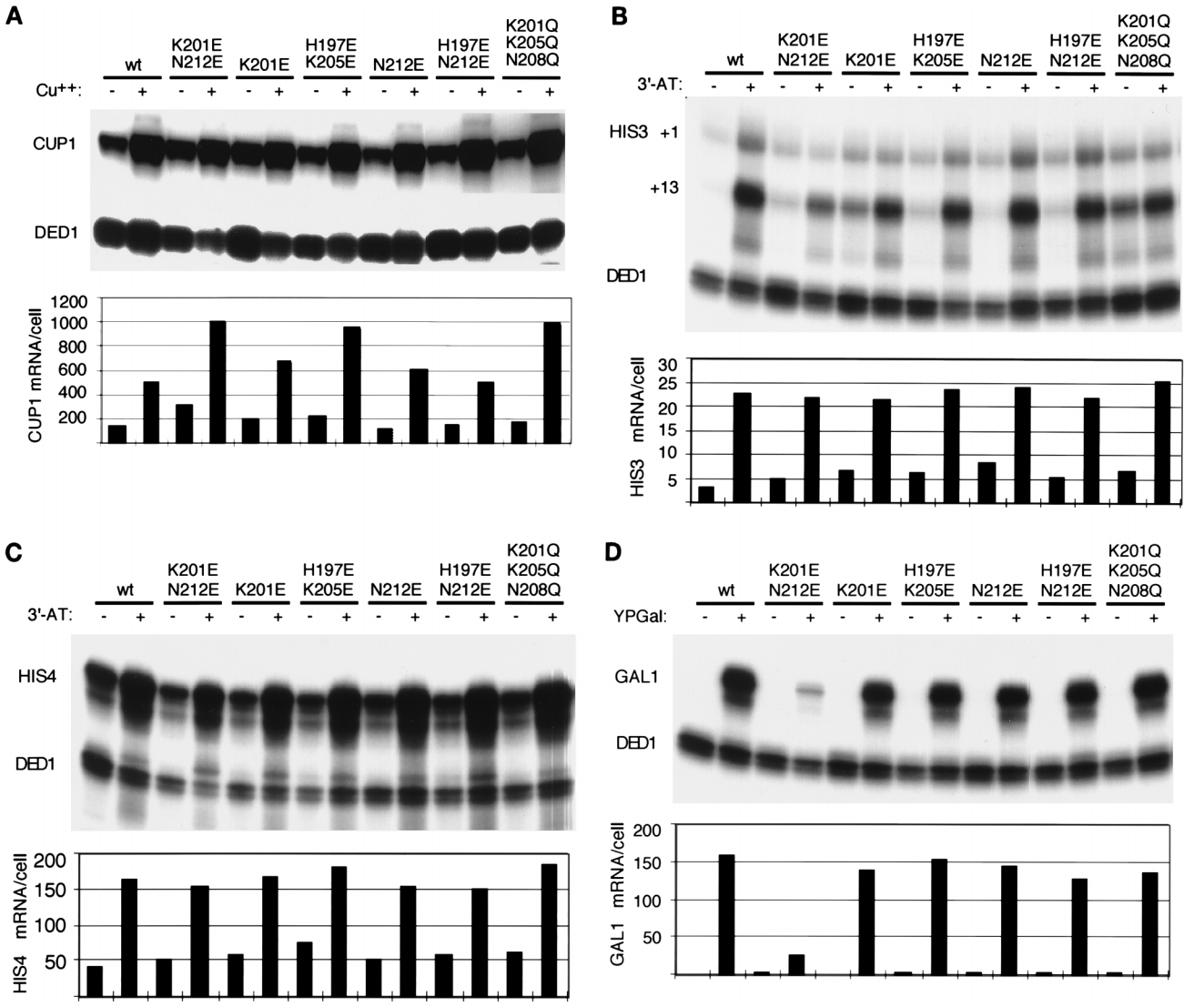

FIG. 6. Activated transcription at naturally inducible promoters in TFIIB mutant strains. (A) Ace1-dependent activation of the *CUP1* gene. The high number of *CUP1* mRNA molecules per cell reflects multiple copies of the *CUP1* gene. The part of the autoradiogram representing *DED1* transcription was exposed for a longer time than that representing *CUP1* transcription. (B) Gcn4-dependent activation of the wild-type *HIS3* gene. (C) Gcn4-dependent activation of the *HIS4* gene. (D) Gal4-dependent activation of the *GAL1* gene. wt, wild type; 3′-AT, aminotriazole.

about twofold in the K201E/N212E background as compared to results for the wild type when cells are grown in galactose medium (Fig. 8). Previous studies indicate that, due to cooperative Gal4 action at the *GAL1* promoter, a twofold decrease in *GAL4* expression results in a fivefold decrease in *GAL1* expression (17). Our results agree closely with these previous findings, suggesting that the defects in Gal4-dependent activation conferred by the K201E/N212E derivative of TFIIB are primarily (and possibly entirely) attributable to an effect on expression of the *GAL4* gene. In addition, this TFIIB mutant strain shows reduced *GAL4* levels when cells are grown in glucose medium (Fig. 8A). This effect in glucose medium, though not understood, provides additional evidence that the K201E/N212E mutant affects *GAL4* transcription.

DISCUSSION

TFIIB is not generally limiting for transcriptional activation in vivo. By definition, a component of a chemical or biological process is limiting if small decrements in its functional concentration or activity decrease the output of the process. Thus, even if an individual component is absolutely required for a process, it is not limiting if large decreases in its activity do not significantly affect the overall output. In this study, we have addressed whether TFIIB is limiting for transcriptional activation in a physiological setting by analyzing mutants with biochemically defined deficits in interactions with the VP16 acidic activation domain and promoter DNA. The use of such mutants addresses two issues: whether the interactions identified in vitro are relevant for recruiting TFIIB to the preinitiation complex in vivo and whether recruitment of TFIIB is limiting for transcriptional activation in vivo.

Our results indicate that TFIIB mutants with severe defects in both VP16 and DNA interactions are generally competent for transcriptional activation in vivo. These mutants behave similarly to TFIIB when assayed on several natural and artificial promoters stimulated by a variety of acidic activators.

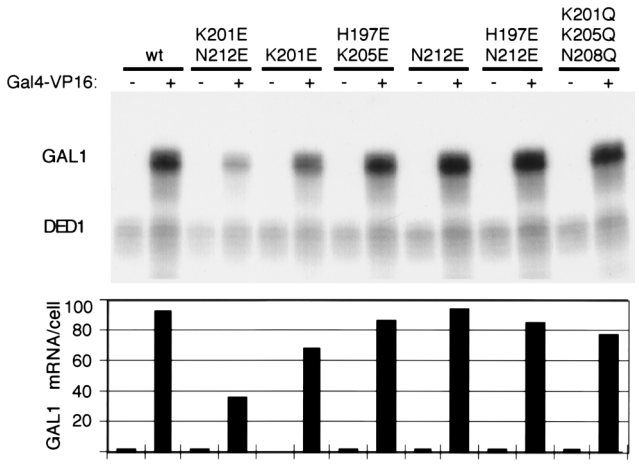

FIG. 7. VP16-dependent activation in TFIIB mutant strains. *GAL1* mRNA levels were measured in TFIIB strains bearing either vector or the Gal4-VP16 activator. wt, wild type.

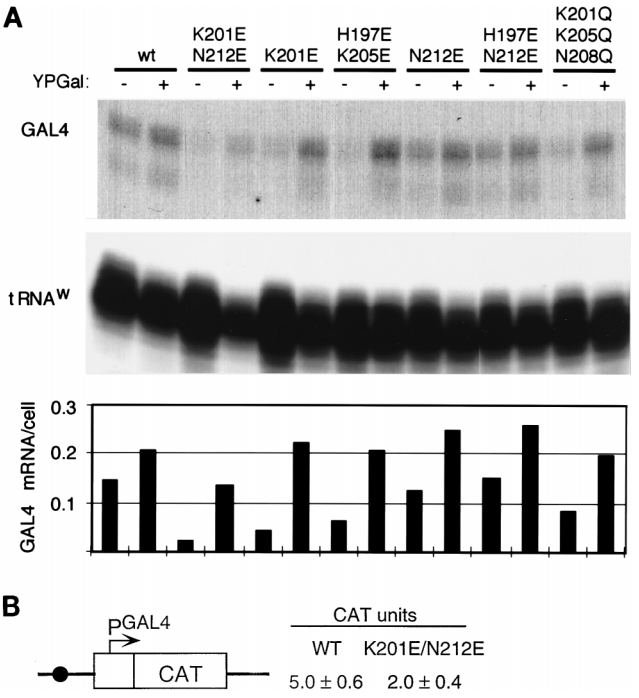

FIG. 8. *GAL4* expression in TFIIB mutant strains. (A) *GAL4* mRNA levels were measured in TFIIB strains by S1 nuclease analysis under repressing (YPD) or activating (YP plus 2% galactose and 0.1% glucose) conditions. wt, wild type. (B) *GAL4* expression was also monitored by using a *GAL4-CAT* fusion and determining the level of CAT activity. WT, wild type.

Furthermore, the K201Q/K205Q/N208Q derivative, which has severe biochemical defects, grows at near-wild-type rates, strongly suggesting that transcription from many natural yeast promoters is unaffected. These TFIIB mutants are synthetically lethal with TBP mutants impaired for the TFIIB interaction, indicating that recruitment of the TFIIB mutants into the preinitiation complex is affected in vivo.

The growth phenotypes of the TFIIB mutants and the effect of the K201E/N212E derivative on *GAL4* transcription provide strong evidence that recruitment of TFIIB is limiting at some promoters in the mutant strains. Thus, even under conditions where TFIIB is artificially made to be limiting at a subset of promoters by virtue of mutations, there is little effect on a range of activated promoters. This argues that TFIIB recruitment is not generally a limiting step for transcriptional activation in wild-type cells. This conclusion is consistent with and significantly extends our previous observation that the TBP-TFIIB interaction is not generally limiting for transcriptional activation in yeast (34).

Because TFIIB is generally required for Pol II transcription (39), the mutant TFIIB derivatives must be sufficiently stabilized at promoters to support high levels of transcription. TFIIB interactions with TAFs, TFIIF, and Pol II (15, 16, 36) might compensate for a defective TFIIB-DNA interaction in vivo. In addition, if TFIIB is recruited to the promoter as a component of the holoenzyme, contacts between other components of the holoenzyme such as TFIIF or TFIIE and various TAFs (14, 21, 50) may be adequate for holoenzyme, and hence TFIIB, recruitment. Finally, the mutant TFIIB derivatives might be stabilized at promoters simply because TFIIB might be present in a sufficiently high concentration and excess over the number of yeast promoters to saturate ternary complex formation. In this regard, there are approximately 20,000 TFIIB molecules/cell (64), which is significantly higher than the 6,000 yeast promoters, and a high TFIIB concentration can suppress the transcriptional defects in vitro of TBP mutants defective for interacting with TFIIB (56).

Yeast promoters differ greatly in their enhancer, TATA, and initiator elements as well as in other aspects which are poorly understood. This diversity makes it very likely that promoters will differ with respect to which of the basic transcription factors are limiting for transcription. Although the activity of most promoters appears to be remarkably resistant to large decreases in TFIIB function, the reduced level of *GAL4* transcription conferred by the K201E/N212E derivative of TFIIB suggests that the *GAL4* promoter is particularly sensitive to TFIIB function. Although the molecular basis of this promoter specificity for TFIIB function is unknown, it is noteworthy that the *GAL4* promoter is extremely weak. Further, the *GAL4* promoter is unusual in that it does not appear to contain a functional TATA element, nor can the essential promoter elements be replaced by a consensus TATA element (18).

TFIIB-VP16 interaction defects in vitro do not correlate with loss of transcriptional activation in vivo. In vitro, the VP16 activation domain interacts with numerous components of the Pol II transcription machinery, such as TBP (23, 54), TFIIA (43), TFIIB (38), TFIIH (62), and TAFs (16). Which, if any, of these interactions are physiologically significant? Our biochemical results indicate that yeast TFIIB is qualitatively and quantitatively similar to human TFIIB with respect to interaction with the VP16 activation domain. Nevertheless, while VP16 interaction mutants of human TFIIB do not support activated transcription in vitro (48), the yeast analogs do not generally affect the response to acidic activators in vivo. Thus, the strength of the interaction between TFIIB and the VP16 activation domain in vitro does not significantly affect the level of transcriptional activation in vivo.

One interpretation of these results is that the observed physical interaction between TFIIB and the VP16 activation domain might be physiologically irrelevant. Binding of TFIIB to the TBP-TATA element complex buries about 1,200-Å^2 of TFIIB surface (41), much of which is protected by the VP16 activation domain in protease footprinting experiments (22). Furthermore, the K201Q/K205Q/N208Q derivative of yeast TFIIB binds poorly to the VP16 activation domain in solution, even though all of the mutated residues lie on the DNA inter-

action surface of TFIIB. These considerations suggest that the DNA-binding and VP16 interaction surfaces of TFIIB significantly overlap, such that the VP16 activation domain might not be able to interact with TFIIB when it is bound to the promoter. The physiological relevance of in vitro interactions with the VP16 activation domain has also previously been questioned because of the existence of TBP mutants that fail to interact with the VP16 activation domain yet support transcriptional activation in mammalian cells (57).

An alternative explanation is that the VP16 activation domain interacts with multiple targets, such that the interaction with TFIIB is not essential for transcriptional enhancement (55). A similar argument has been advanced to account for why TAFs are essential for transcriptional activation in vitro but are generally dispensable in yeast cells (39, 59). In this view, the strong correlation between the functional quality of an activation domain and the strength of interaction with TBP and TFIIB (40, 60) may reflect a more general ability of activation domains to interact with a variety of components of the Pol II machinery rather than a specific relationship to a physiological target. Finally, it should be noted that activation defects of human TFIIB mutants in vitro (48) might not be due to the reduced interaction with the VP16 activation domain itself but rather to weakened interactions with the promoter or other factors in the preinitiation complex that mediate the effect of the VP16 activation domain.

Comparison between transcription experiments in vitro and in vivo. There are several explanations for the apparent contrast between the ability of our yeast TFIIB mutants to generally support transcriptional activation in vivo and the observed activation defects of the analogous human TFIIB mutants in vitro (48). First, the experimental conditions in vitro are likely to be quite different from those inside yeast cells. In particular, in vitro experiments utilize nonchromatin DNA templates and nonphysiological concentrations of components of the Pol II machinery, both of which are likely to affect limiting steps for transcriptional activation. Second, although the process of transcriptional activation is highly conserved among eukaryotes, the possibility of species-specific differences between yeast and human can not be excluded. Third, analysis of similar yeast TFIIB mutants in vitro under different experimental conditions indicates that a significant defect in TBP-TFIIB-TATA complex formation does not necessarily affect transcriptional activation by the VP16 domain in vitro (3). Furthermore, TFIIB mutants that more severely disrupt TBP-TFIIB-TATA complex formation show reduced levels of basal transcription in vitro but show an equivalent VP16-dependent enhancement of transcription (3). In this regard, the human TFIIB mutants are also defective in basal transcription, and there is disagreement about the effects on activator-dependent transcription (19, 48). Whatever the explanation for the results of these in vitro transcription experiments, our results strongly argue that recruitment of TFIIB is not generally a limiting step for acidic activators in yeast cells.

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REFERENCES

1. **Apone, L. M., C. A. Virbasius, J. C. Reese, and M. R. Green.** 1996. Yeast $TAF_{II}90$ is required for cell-cycle progression through G_2/M but not for general transcription activation. Genes Dev. **10:**2368–2380.
2. **Arndt, K. M., S. Ricupero-Hovasse, and F. Winston.** 1995. TBP mutants defective in activated transcription *in vivo*. EMBO J. **14:**1490–1497.
3. **Bangur, C. S., T. S. Pardee, and A. S. Ponticelli.** 1997. Mutational analysis of the D1/E1 core helices and the conserved N-terminal region of yeast transcriptional factor IIB (TFIIB): identification of an N-terminal mutant that stablizes TATA-binding protein–TFIIB–DNA complexes. Mol. Cell. Biol. **17:**6784–6793.
4. **Barberis, A., J. Pearlberg, N. Simkovich, S. Farrell, P. Reinagel, C. Bamdad, G. Sigal, and M. Ptashne.** 1995. Contact with a component of the polymerase II holoenzyme suffices for gene activation. Cell **81:**359–368.
5. **Berger, S. L., B. Pina, N. Silverman, G. A. Marcus, J. Agapite, J. L. Regier, S. J. Triezenberg, and L. Guarente.** 1992. Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell **70:**251–265.
6. **Boeke, J. D., J. Trueheart, G. Natsoulis, and G. R. Fink.** 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:**164–175.
7. **Bryant, G. O., L. S. Martel, S. K. Burley, and A. J. Berk.** 1996. Radical mutations reveal TATA-box binding protein surfaces required for activated transcription *in vivo*. Genes Dev. **10:**2491–2504.
8. **Chatterjee, S., and K. Struhl.** 1995. Connecting a promoter-bound protein to the TATA-binding protein overrides the need for a transcriptional activation region. Nature **374:**820–822.
9. **Chen, W., and K. Struhl.** 1988. Saturation mutagenesis of a yeast *his3* TATA element: genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. USA **85:**2691–2695.
10. **Colgan, J., H. Ashali, and J. L. Manley.** 1995. A direct interaction between a glutamine-rich activator and the N terminus of TFIIB can mediate transcriptional activation in vivo. Mol. Cell. Biol. **15:**2311–2320.
11. **Colgan, J., S. Wampler, and J. L. Manley.** 1993. Interaction between a transcriptional activator and transcription factor IIB *in vivo*. Nature **362:**549–553.
12. **Cormack, B. P., M. Strubin, A. S. Ponticelli, and K. Struhl.** 1991. Functional differences between yeast and human TFIID are localized to the highly conserved region. Cell **65:**341–348.
13. **Couto, G., N. Klages, and M. Strubin.** 1997. Synergistic and promoter-selective activation of transcription by recruitment of TFIID and TFIIB. Proc. Natl. Acad. Sci. USA **94:**8036–8041.
14. **Dubrovskaya, V., A.-C. Lavigne, I. Davidson, J. Acker, A. Staub, and L. Tora.** 1996. Distinct domains of $hTAF_{II}100$ are required for functional interaction with transcription factor TFIIFβ (RAP30) and incorporation into the TFIID complex. EMBO J. **15:**3702–3712.
15. **Fang, S. M., and Z. F. Burton.** 1996. RNA polymerase II-associated protein (RAP) 74 binds transcription factor (TF) IIB and blocks TFIIB-RAP30 binding. J. Biol. Chem. **271:**11703–11709.
16. **Goodrich, J. A., T. Hoey, C. J. Thut, A. Admon, and R. Tjian.** 1993. Drosophila $TAF_{II}40$ interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell **75:**519–530.
17. **Griggs, D. W., and M. Johnston.** 1991. Regulated expression of the GAL4 activator gene in yeast provides a sensitive switch for glucose repression. Proc. Natl. Acad. Sci. USA **88:**8597–8601.
18. **Griggs, D. W., and M. Johnston.** 1993. Promoter elements determining weak expression of the *GAL4* regulatory gene of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**4999–5009.
19. **Gupta, R., A. Emili, G. Pan, H. Xiao, M. Shales, J. Greenblatt, and C. J. Ingles.** 1996. Characterization of the interaction between the acidic activation domain of VP16 and the RNA polymerase II initiation factor TFIIB. Nucleic Acids Res. **24:**2324–2330.
20. **Ha, I., W. S. Lane, and D. Reinberg.** 1991. Cloning of a human gene encoding the general transcription initiation factor IIB. Nature **352:**689–695.
21. **Hisatake, K., T. Ohta, R. Takada, M. Guermah, M. Horikoshi, Y. Nakatani, and R. G. Roeder.** 1995. Evolutionary conservation of human TATA-binding-polypeptide-associated factors TAFII31 and TAFII80 and interactions of TAFII80 with other TAFs and with general transcription factors. Proc. Natl. Acad. Sci. USA **92:**8195–8199.
22. **Hori, R., S. Pyo, and M. Carey.** 1995. Protease footprinting reveals a surface on transcription factor TFIIB that serves as an interface for activators and coactivators. Proc. Natl. Acad. Sci. USA **92:**6047–6051.
23. **Ingles, C. J., M. Shales, W. D. Cress, S. J. Triezenberg, and J. Greenblatt.** 1991. Reduced binding of TFIID to transcriptionally compromised mutants of VP16. Nature **351:**588–590.
24. **Iyer, V., and K. Struhl.** 1995. Mechanism of differential utilization of the *his3* T_R and T_C TATA elements. Mol. Cell. Biol. **15:**7059–7066.
25. **Iyer, V., and K. Struhl.** 1995. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic structure. EMBO J. **14:**2570–2579.
26. **Iyer, V., and K. Struhl.** 1996. Absolute mRNA levels and transcriptional

initiation rates in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **93:** 5208–5212.
27. **Jiang, Y. W., and D. J. Stillman.** 1992. Involvement of the *SIN4* global transcriptional regulator in the chromatin structure of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**4503–4514.
28. **Keaveney, M., and K. Struhl.** Unpublished results.
29. **Kim, T. K., and R. G. Roeder.** 1994. Proline-rich activator CTF1 targets the TFIIB assembly step during transcriptional activation. Proc. Natl. Acad. Sci. USA **91:**4170–4174.
30. **Kim, Y.-J., S. Bjorklund, Y. Li, M. H. Sayre, and R. D. Kornberg.** 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:**599–608.
31. **Klages, N., and M. Strubin.** 1995. Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo. Nature **374:**822–823.
32. **Koleske, A. J., and R. A. Young.** 1994. An RNA polymerase II holoenzyme responsive to activators. Nature **368:**466–469.
33. **Lee, M., and K. Struhl.** 1995. Mutations on the DNA-binding surface of TBP can specifically impair the response to acidic activators *in vivo*. Mol. Cell. Biol. **15:**5461–5469.
34. **Lee, M., and K. Struhl.** 1997. A severely defective TATA-binding protein–TFIIB interaction does not preclude transcriptional activation *in vivo*. Mol. Cell. Biol. **17:**1336–1345.
35. **Lee, S., and S. Hahn.** 1995. Model for binding of transcription factor TFIIB to the TBP-DNA complex. Nature **376:**609–612.
36. **Li, Y., P. M. Flanagan, H. Tschochner, and R. D. Kornberg.** 1994. RNA polymerase II initiation factor interactions and transcription start site selection. Science **263:**805–807.
37. **Lin, Y.-S., and M. R. Green.** 1991. Mechanism of action of an acidic transcriptional activator *in vitro*. Cell **64:**971–981.
38. **Lin, Y.-S., I. Ha, E. Maldonado, D. Reinberg, and M. R. Green.** 1991. Binding of general transcription factor TFIIB to an acidic activating region. Nature **353:**569–571.
39. **Moqtaderi, Z., Y. Bai, D. Poon, P. A. Weil, and K. Struhl.** 1996. TBP-associated factors are not generally required for transcriptional activation in yeast. Nature **382:**188–191.
40. **Nerlov, C., and E. B. Ziff.** 1995. CCAAT/enhancer binding protein-α amino acid motifs with dual TBP and TFIIB binding ability co-operate to activate transcription in both yeast and mammalian cells. EMBO J. **14:**4318–4328.
41. **Nikolov, D. B., H. Chen, E. D. Halay, A. A. Usheva, K. Hisatake, D. K. Lee, R. G. Roeder, and S. K. Burley.** 1995. Crystal structure of a TFIIB-TBP-TATA-element ternary complex. Nature **377:**119–128.
42. **Orphanides, G., T. Lagrange, and D. Reinberg.** 1996. The general initiation factors of RNA polymerase II. Genes Dev. **10:**2657–2683.
43. **Ozer, J., P. A. Moore, A. H. Bolden, A. Lee, C. A. Rosen, and P. M. Lieberman.** 1994. Molecular cloning of the small (γ) subunit of human TFIIA reveals functions critical for activated transcription. Genes Dev. **8:**2324–2335.
44. **Pinto, I., D. E. Ware, and M. Hampsey.** 1992. The yeast *SUA7* gene encodes a homolog of human transcription factor TFIIB and is required for normal start site selection in vivo. Cell **68:**977–988.
45. **Ptashne, M., and A. Gann.** 1997. Transcriptional activation by recruitment. Nature **386:**569–577.
46. **Roberts, S. G., B. Choy, S. S. Walker, Y. S. Lin, and M. R. Green.** 1995. A role for activator-mediated TFIIB recruitment in diverse aspects of transcriptional regulation. Curr. Biol. **5:**508–516.
47. **Roberts, S. G., and M. R. Green.** 1994. Activator-induced conformational change in general transcription factor TFIIB. Nature **371:**717–720.
48. **Roberts, S. G., I. Ha, E. Maldonado, D. Reinberg, and M. R. Green.** 1993. Interaction between an acidic activator and transcription factor TFIIB is required for transcriptional activation. Nature **363:**741–744.
49. **Roeder, R. G.** 1996. The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem. Sci. **21:**327–335.
50. **Ruppert, S., and R. Tjian.** 1995. $TAF_{II}250$ interacts with RAP74: implications for RNA polymerase II initiation. Genes Dev. **9:**2747–2755.
51. **Shaw, S. P., J. Wingfield, M. J. Dorsey, and J. Ma.** 1996. Identifying a species-specific region of yeast TFIIB in vivo. Mol. Cell. Biol. **16:**3651–3657.
52. **Stargell, L. A., and K. Struhl.** 1995. The TBP-TFIIA interaction in the response to acidic activators *in vivo*. Science **269:**75–78.
53. **Stargell, L. A., and K. Struhl.** 1996. A new class of activation-defective TATA-binding protein mutants: evidence for two steps of transcriptional activation in vivo. Mol. Cell. Biol. **16:**4456–4464.
54. **Stringer, K. F., C. J. Ingles, and J. Greenblatt.** 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. Nature **345:**783–786.
55. **Struhl, K.** 1996. Chromatin structure and RNA polymerase II connection: implications for transcription. Cell **84:**179–182.
56. **Tang, H., X. Sun, D. Reinberg, and R. Ebright.** 1996. Protein-protein interactions in eukaryotic transcription initiation: structure of the preinitiation complex. Proc. Natl. Acad. Sci. USA **93:**1119–1124.
57. **Tansey, W. P., and W. Herr.** 1995. The ability to associate with activation domains *in vitro* is not required for the TATA box-binding protein to support activated transcription *in vivo*. Proc. Natl. Acad. Sci. USA **92:**10550–10554.
58. **Tansey, W. P., and W. Herr.** 1997. Selective use of TBP and TFIIB revealed by a TATA-TBP-TFIIB array with altered specificity. Science **275:**829–831.
59. **Walker, S. S., J. C. Reese, L. M. Apone, and M. R. Green.** 1996. Transcription activation in cells lacking TAF_{II}s. Nature **382:**185–188.
60. **Wu, Y. B., R. J. Reece, and M. Ptashne.** 1996. Quantitation of putative activator-target affinities predicts transcriptional activating potentials. EMBO J. **15:**3951–3963.
61. **Xiao, H., J. D. Friesen, and J. T. Lis.** 1995. Recruiting TATA-binding protein to a promoter: transcriptional activation without an upstream activator. Mol. Cell. Biol. **15:**5757–5761.
62. **Xiao, H., A. Pearson, B. Coulombe, R. Truant, S. Zhang, J. L. Regier, S. J. Triezenberg, D. Reinberg, O. Flores, C. J. Ingles, and J. Greenblatt.** 1994. Binding of basal transcription factor TFIIH to the acidic activation domains of VP16 and p53. Mol. Cell. Biol. **14:**7013–7024.
63. **Yamashita, S., K. Hisatake, T. Kokubo, K. Doi, R. G. Roeder, M. Horikoshi, and Y. Nakatani.** 1993. Transcription factor TFIIB sites important for interaction with promoter-bound TFIID. Science **261:**463–466.
64. **Young, R. A.** Personal communication.