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Identifying a Species-Specific Region of Yeast TFIIB In Vivo

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The general transcription factor IIB (TFIIB) is required for RNA polymerase II transcription in eukaryotes. It provides a physical link between the TATA-binding protein (TBP) and the RNA polymerase and is a component previously suggested to respond to transcriptional activators in vitro. In this report, we compare the yeast (*Saccharomyces cerevisiae*) and human forms of the protein in yeast cells to study their functional differences. We demonstrate that human TFIIB fails to functionally replace yeast TFIIB in yeast cells. By analyzing various human-yeast hybrid TFIIB molecules, we show that a 14-amino-acid region at the amino terminus of the first repeat of yeast TFIIB plays an important role in determining species specificity in vivo. In addition, we identify four amino acids in this region that are critical for an amphipathic helix unique to yeast TFIIB. By site-directed mutagenesis analyses we demonstrate that these four amino acids are important for yeast TFIIB's activity in vivo. Finally, we show that mutations in the species-specific region of yeast TFIIB can differentially affect the expression of genes activated by different activators in vivo. These results provide strong evidence suggesting that yeast TFIIB is involved in the process of transcriptional activation in living cells.

RNA polymerase II transcription in eukaryotes requires a class of proteins called general transcription factors (39). These proteins enable RNA polymerase II to recognize gene promoters and participate in the assembly of preinitiation complexes. One of these proteins, the general transcription factor IIB (TFIIB), provides a physical link between the TATA-binding protein (TBP) and RNA polymerase II within the preinitiation complex (3, 7, 18, 58). According to a previously suggested stepwise pathway (6), the first step of preinitiation complex formation is the binding of TBP to DNA. Then TFIIB enters the complex, followed by RNA polymerase II and other general transcription factors. As an integral component of the preinitiation complex, TFIIB also interacts with at least two other general transcription factors, TFIIF and a TBP-associated factor (TAF40) (16, 18). More recent biochemical studies suggest that TFIIB is part of the RNA polymerase II holoenzyme (31, 43, 55).

One of the central problems in molecular biology is to understand how transcriptional activators stimulate gene expression in eukaryotes. At the step of transcription initiation, activators could in principle either increase the number of preinitiation complexes by recruiting the general transcription factors or change the quality of preinitiation complexes by increasing their stability or inducing conformational changes. Many general transcription factors (60), including TBP (23, 50), TAFs (16, 22), TFIIA (44), TFIIB (2, 10, 26, 37, 49), TFIIF (24), and TFIIH (57), have been shown to interact with various transcriptional activators in vitro. In particular, a series of biochemical experiments strongly suggest that TFIIB plays an important role in transcriptional activation in vitro. First, the ability of an activator to interact with TFIIB correlates with its ability to activate transcription (37). In addition, mutant TFIIB molecules that are defective in interacting with activators fail to support activated transcription in vitro, while basal transcription remains unaffected (48). Finally, transcriptional activators can stabilize the step in which TFIIB joins the preinitiation complex, a step that appears slow and/or inefficient in the absence of an activator (36).

Despite these biochemical experiments suggesting the importance of TFIIB in transcriptional activation, it remains to be determined whether and how TFIIB participates in transcriptional activation in living cells. To gain insights into functional domains of TFIIB and their possible roles in transcriptional activation in vivo, we compared human and yeast forms of the protein in yeast cells. Although TFIIB is highly conserved among humans, rats, *Xenopus laevis*, and *Drosophila melanogaster* (17, 21, 40, 53, 54), there is only 35% amino acid identity between yeast TFIIB (yTFIIB) (46) and human TFIIB (hTFIIB). Sequence analysis suggests that eukaryotic TFIIBs share several putative structural motifs, including a zinc finger motif at the amino terminus, two imperfect repeats at the carboxy-terminal portion, and a putative helix region between the two repeats (17, 46). In this report, we first demonstrate that hTFIIB fails to functionally replace yTFIIB in yeast cells. By analyzing various hybrid TFIIB molecules, we show that a region at the amino terminus of the first repeat of yTFIIB provides important species-specific function in vivo. We further provide evidence suggesting that this region of yTFIIB participates in the process of transcriptional activation in living cells.

MATERIALS AND METHODS

Strain construction and the plasmid shuffle system. The plasmid shuffle system was performed as previously described (5). Briefly, the entire coding sequence of the endogenous *yTFIIB* gene in *Saccharomyces cerevisiae* S150B (**a** *leu2-3 his3 trp1-289 ura3-52*) (56) was deleted by replacement with the *LEU2* gene, while the cell viability was maintained by a *URA3* plasmid expressing the wild-type yTFIIB (pDW5462) (46). *TRP1* plasmids expressing various hybrid TFIIB molecules were then transformed into this yeast strain, and *TRP1*$^+$ *URA3*$^+$ transformants were grown in liquid media lacking tryptophan and uracil. Serially diluted (10-fold) liquid cultures were spotted on either minimal plates lacking tryptophan and uracil or plates containing 5-fluoroorotic acid (5-FOA) and incubated at 30°C.

Hybrid TFIIB genes. pMA1210A, a *TRP1* 2μm plasmid expressing the wild-type yTFIIB from the *ADH1* promoter, was constructed from pADNS (11) and pDW5462 (46). All the hybrid genes were first constructed by PCR and then transferred to the backbone of pMA1210A. The gene encoding SSP, which was generated from the inactive derivative *SAC1*, was verified by DNA sequencing.

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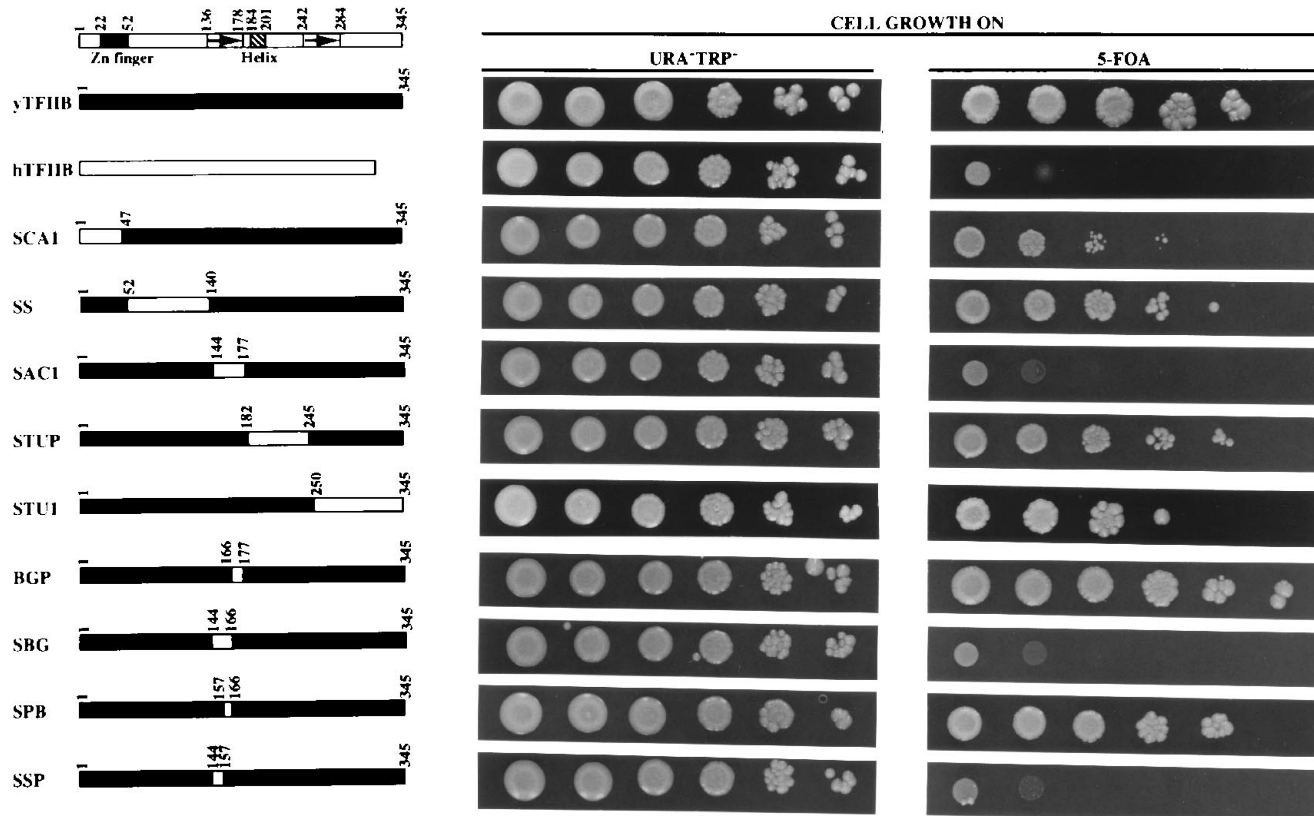

FIG. 1. Analysis of human-yeast hybrid TFIIB molecules in yeast cells. Shown are schematic diagrams of the hybrid TFIIB molecules and their abilities to function in yeast cells. Serially (10 times) diluted yeast cultures were spotted on plates containing 5-FOA, and the ability of cells to grow reflects the ability of hybrid proteins to functionally replace yTFIIB in vivo (see Materials and Methods for further details). The same serially diluted cultures were also spotted on plates lacking 5-FOA, tryptophan, and uracil to estimate the total number of cells analyzed. The structural motifs diagrammed here are those described by Pinto et al. (46). All exchanges were generated according to the sequence alignment generated by Pinto et al. (46), and therefore, only yTFIIB amino acid positions are indicated for simplicity. Because there are amino acid identities at the fusion junctions, the exchanged positions shown here reflect the first and last amino acids that differ between yTFIIB and hTFIIB rather than the actual junctions of DNA sequences. For example, residues at positions 48 to 51 of yTFIIB are identical to the corresponding residues of hTFIIB, and they are not included in either SCA1 or SS. It should be noted that according to the recently reported hTFIIB structure (1, 41), as predicted previously (17), the two repeats of hTFIIB are longer than those diagrammed here (arrows), with the first repeat of hTFIIB covering the putative helix. In addition, this putative helix is more carboxy terminally positioned in hTFIIB than it is in yTFIIB (17, 46). Finally, another putative helix located before the first repeat of hTFIIB, as predicted by Yamashita et al. (58), is not shown for yTFIIB.

A site-directed mutagenesis procedure based on PCR was used to generate the genes for loss-of-function and gain-of-function experiments whose results are shown in Fig. 4. For experiments whose results are shown in Fig. 6, the *yTFIIB* promoter sequence was first inserted into pPC86, an ARS-CEN plasmid (9), to create the vector BLC0. The *yTFIIB* genes were then transferred to this vector and analyzed in the plasmid shuffle system.

Immunoblotting analysis. For immunoblotting assays, an oligonucleotide encoding the hemagglutinin tag (MAYPYDVPDYAH) was fused to the first codon of the *TFIIB* genes. Yeast cells transformed with these modified plasmids were grown in 50 ml of synthetic media lacking uracil and tryptophan with 2% glucose. Proteins extracted from $\sim 10^7$ cells were separated on sodium dodecyl sulfate-polyacrylamide gels, transferred to nitrocellulose, blotted with an antihemagglutinin monoclonal antibody (HA.11 [BabCo]; 1:300 final dilution), and visualized by enhanced chemiluminescence (Amersham). A monoclonal antibody (C4, kindly provided by J. Lessard's laboratory; 6-μg/ml final concentration) against the conserved region of actin (33) was used to determine the actin level as an internal control.

β-Galactosidase assays. Yeast strains bearing either the wild-type or mutant YR1m4 yTFIIB were generated by 5-FOA selection and transformed with reporter genes carried on *URA3* plasmids. Yeast transformants were grown in glucose (2%) liquid culture lacking uracil until saturation, diluted in galactose (2%) liquid medium, incubated until the optical density at 600 nm reached 1 to 1.5, and assayed for β-galactosidase activity as previously described (38, 59).

RNA analysis. Yeast cells bearing either the wild-type or mutant YR1m4 yTFIIB were grown in 20 ml of YPD medium. Total RNA was isolated and primer extension analysis was performed as described previously (38).

RESULTS

hTFIIB fails to complement yTFIIB in vivo. We used a yeast plasmid shuffle system (5) to determine whether hTFIIB can functionally replace yTFIIB in vivo. First, the endogenous *yTFIIB* gene (*SUA7*) was deleted while cell viability was maintained by a copy of the wild-type *yTFIIB* gene carried on a plasmid (pDW5462) (46). A second plasmid carrying another *TFIIB* gene to be tested was then transformed into the yeast strain. Finally, pDW5462, which carries a yeast *URA3* gene, was eliminated from yeast cells with 5-FOA, a chemical toxic to cells expressing the *URA3* gene. Thus, the ability of yeast cells to grow on 5-FOA plates reflects the in vivo activity of TFIIB expressed from the second plasmid. While yeast cells expressing yTFIIB grew well on 5-FOA plates as expected, those expressing hTFIIB completely failed to survive the 5-FOA selection (Fig. 1). These results suggest that yTFIIB and hTFIIB are specific to their respective species in vivo.

The amino terminus of the first repeat of yTFIIB provides important species-specific function. To determine which putative structural motif or other region of yTFIIB determines the species specificity, we generated hybrid genes encoding yTFIIB

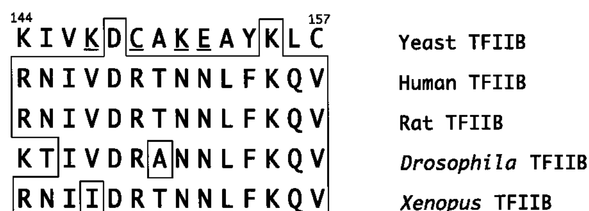

FIG. 2. Sequence comparison. The 14-amino-acid species-specific region of yTFIIB (residues 144 to 157) is aligned with the corresponding sequences from other eukaryotic TFIIBs. The four critical amino acids of yTFIIB that represent the major differences from hTFIIB are underlined (see Fig. 3 and the text for further details).

derivatives with various motifs and/or regions replaced by their corresponding human sequences (see the legend to Fig. 1 for details). The following regions were tested individually: (i) the amino terminus, including the zinc finger motif (SCA1); (ii) the region between the zinc finger motif and the first repeat (SS); (iii) the first repeat (SAC1); (iv) the region between the two repeats, which includes the putative helix (STUP); and (v) the entire carboxy terminus, which includes the second repeat (STU1). While yeast cells bearing SAC1 completely failed to grow on 5-FOA plates, those bearing each of the other derivatives all survived this selection to various extents (Fig. 1). These experiments demonstrate that each of the individual regions of yTFIIB, except the first repeat, can be functionally replaced by its corresponding human sequence.

The experiments described thus far clearly demonstrate that the most significant difference between yTFIIB and hTFIIB resides within the first repeat, and therefore we focused our further analysis on this region. However, it should be noted that other regions of yTFIIB, which are obviously less important in determining species specificity, may nonetheless provide additional species-specific functions in vivo. We have generated hybrid genes encoding yTFIIB derivatives with multiple regions replaced by their corresponding human sequences. While cells bearing some of these derivatives grew well on 5-FOA plates, cells bearing others did not (unpublished results). In addition, some of the derivatives bearing individual exchanged structural motifs (e.g., STUP and SCA1), though functional in yeast cells at normal temperature (30°C), conferred a moderate temperature-sensitive phenotype (data not shown). It is worth noting that the species specificity of TBP has been suggested to be determined by multiple regions (12, 14).

To further narrow down the region responsible for determining species specificity of yTFIIB, we exchanged shorter segments in the first repeat of yTFIIB with hTFIIB. First, we generated hybrid genes encoding TFIIB derivatives with either the amino-terminal (SBG) or the carboxy-terminal (BGP) half of the first repeat of yTFIIB replaced by the corresponding human sequence (Fig. 1). While cells bearing BGP grew well on 5-FOA plates, cells bearing SBG did not grow at all (Fig. 1), demonstrating that the amino-terminal half of the first repeat of yTFIIB is nonexchangeable. We then generated hybrid genes further dividing this nonexchangeable region into the amino-terminal (SSP) and carboxy-terminal (SPB) halves (Fig. 1). While cells containing SPB grew well on 5-FOA plates, cells containing SSP virtually failed to grow on 5-FOA plates (Fig. 1), suggesting that the amino-terminal quarter of the first repeat of yTFIIB is nonexchangeable. A small number of cells bearing SSP eventually grew on 5-FOA plates, but the number of surviving cells was 3 to 4 orders of magnitude lower than that for cells bearing SPB or wild-type yTFIIB (Fig. 1). In addition, the surviving cells bearing SSP grew extremely slowly on both synthetic- and rich-medium plates and exhibited a temperature-sensitive phenotype (Fig. 1 and data not shown).

An amphipathic helix is important for yTFIIB function. The nonexchangeable region in the first repeat of yTFIIB is composed of 14 amino acids (residues 144 to 157). Although this region is highly conserved among TFIIBs from humans, rats, *X. laevis*, and *D. melanogaster* (17, 21, 40, 53, 54), consistent with the previous demonstration that hTFIIB and *Drosophila* TFIIB are functionally interchangeable in vitro (54), only two amino acids are identical between yTFIIB and hTFIIB in this region (Fig. 2). According to the recently published structure of hTFIIB (1, 41), the region corresponding to the species-specific region of yTFIIB is folded into a solvent-exposed helix (designated BH2). It has been suggested (1, 41) that all the eukaryotic TFIIBs isolated thus far may form structures similar to that of hTFIIB, and therefore, the species-specific region in yTFIIB could be similarly folded into a solvent-exposed helix. The yTFIIB helix would be amphipathic, with charged residues (net charge, +2) on one side and hydrophobic residues on the other (Fig. 3). The corresponding human helix has fewer charged residues, though with the same net charge (Fig. 3). In addition, the relative positions of the charged surfaces of the respective helices are slightly different (Fig. 3). We noted that these potential structural differences are mainly contributed by four amino acid residues at positions 147, 149, 151, and 152 that differ between yTFIIB and hTFIIB (underlined in Fig. 2 and 3).

To determine whether the amphipathic helix at the amino terminus of the first repeat of yTFIIB is important for the protein's activity in vivo, we conducted two different analyses by changing the four critical amino acids. In a loss-of-function assay, we generated a mutant *yTFIIB* gene encoding an otherwise wild-type yTFIIB with only these four residues changed to the corresponding human residues (YR1m4) (Fig. 4). The ability of yeast cells bearing this derivative to grow on 5-FOA plates was significantly impaired (Fig. 4). The number of cells surviving 5-FOA selection was 3 orders of magnitude lower than that for cells bearing the wild-type yTFIIB (Fig. 4). In addition, the surviving cells grew noticeably slowly and exhibited a temperature-sensitive phenotype (Fig. 4 and data not shown). These results suggest that these four amino acids of yTFIIB are essential for its activity in vivo. In a gain-of-function assay, we converted the corresponding four human residues to yeast residues in two inactive hybrid derivatives (SBG and SAC1 to HR1m5 and SACm5, respectively) (Fig. 4). Yeast cells bearing these modified hybrid derivatives gained the ability to grow on 5-FOA plates (Fig. 4), indicating that these four

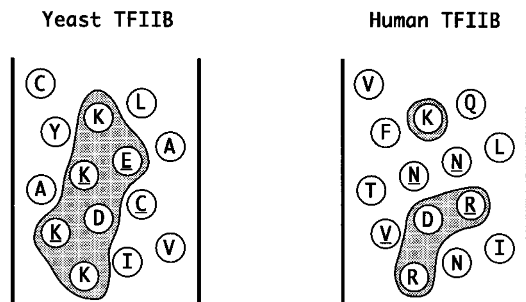

FIG. 3. Amphipathic helices from yTFIIB and hTFIIB. Shaded areas indicate charged surfaces, and the underlined four residues represent the major differences between yTFIIB and hTFIIB. In the recently reported crystal structure of hTFIIB, the human helix shown here represents BH2 (1, 41).

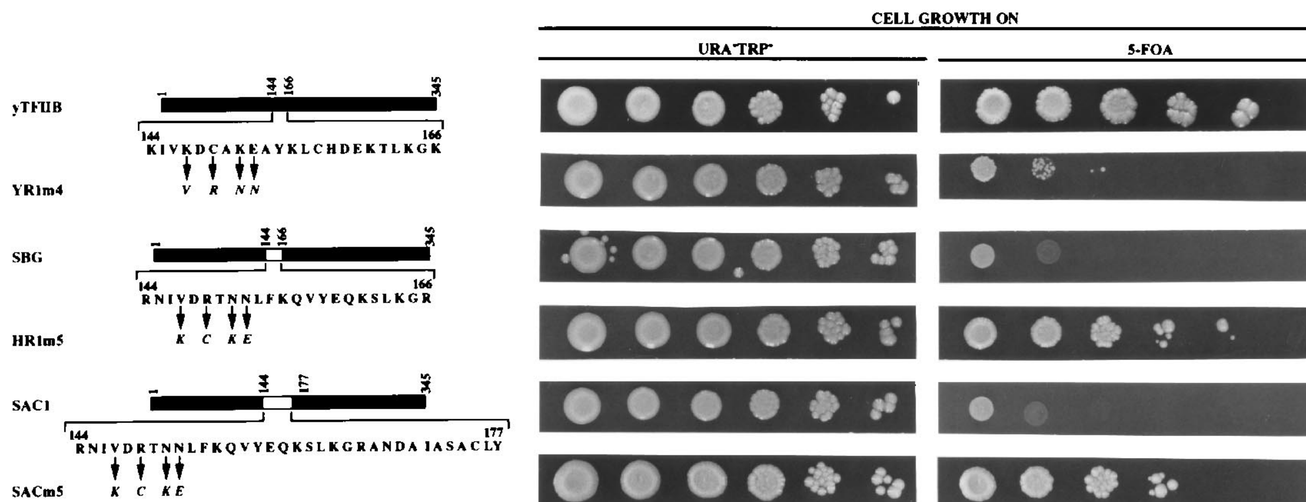

FIG. 4. Loss-of-function and gain-of-function analyses. Mutant *yTFIIB* genes, which were constructed by site-directed mutagenesis, were assayed in a manner similar to that for Fig. 1.

amino acids from yTFIIB are sufficient to confer biological functions to these inactive yeast-human hybrid derivatives. Taken together, our results demonstrate that the four amino acids (Lys-147, Cys-149, Lys-151, and Glu-152), which are important for the amphipathic helix at the amino terminus of the first repeat of yTFIIB, provide essential species-specific functions in vivo. Consistent with our results, one of the temperature-sensitive mutants of yTFIIB isolated by Knaus et al. (29) has a change of cysteine to arginine at amino acid position 149.

TFIIB protein levels in yeast cells. In the experiments whose results are shown in Fig. 1 and 4, all the TFIIB derivatives were expressed from the strong *ADH1* promoter on multicopy plasmids to ensure high protein levels in yeast cells. This would help to avoid the possibility that the observed phenotypes of the TFIIB derivatives were due to protein levels rather than their functional differences in vivo. In an immunoblotting assay (Fig. 5; see Materials and Methods for details), most of these TFIIB proteins were present in yeast cells at comparable levels. These levels were all higher than that of yTFIIB expressed from its own promoter on a single-copy plasmid (data not shown). In this immunoblotting assay, STUP was present at a lower level than the others and a faster-moving band may represent a proteolytic product. It should be emphasized that STUP is a functional TFIIB derivative in yeast cells and that none of the inactive derivatives was accumulated at lower protein levels. These results strongly suggest that the observed phenotypes of the TFIIB proteins were due to their functional differences rather than protein levels.

We also analyzed several key TFIIB proteins when they were expressed at physiological levels. In the experiments whose results are shown in Fig. 6, all the proteins were expressed from the *yTFIIB* promoter on ARS-CEN plasmids. As expected, wild-type yTFIIB expressed from the plasmid was able to complement the endogenous *yTFIIB* gene. A hybrid TFIIB derivative (SBG) with yeast residues 144 to 166 replaced by the corresponding human sequence was completely inactive in yeast cells. Conversion of the four human amino acid residues to yeast residues (HR1m5) restored its in vivo function. These results demonstrate that the TFIIB derivatives expressed at physiological levels behave identically to their respective overexpressed proteins. They further suggest that the observed phenotypes shown in Fig. 1 and 4 reflect the functional differences of the TFIIB derivatives at physiological levels.

Transcription start sites. It has been shown previously that TFIIB is involved in determining transcription start sites (34, 46). Transcription starts about 40 to 120 bp downstream of the TATA box in *S. cerevisiae*, whereas it starts about 30 bp downstream of the TATA box in humans (reviewed in reference 34). Using a primer extension analysis (38), we determined transcription start sites in yeast cells bearing various yTFIIB derivatives either in the presence of the wild-type yTFIIB when the derivatives were nonfunctional or in the absence of yTFIIB when they were sufficiently functional to support cell viability. We did not detect any start sites that were indicative of the human transcription system, i.e., 30 bp downstream of the TATA box (Fig. 7 and data not shown). These results are consistent with those of previous studies demonstrating that the difference between *S. cerevisiae* and other eukaryotes in start site selection is mediated by RNA polymerase II and TFIIB together (34). In these experiments only minor alternations of transcription start sites were detected. For example, the relative intensities of the two major start sites of the *ADH1* gene were slightly altered in cells bearing a mutant yTFIIB

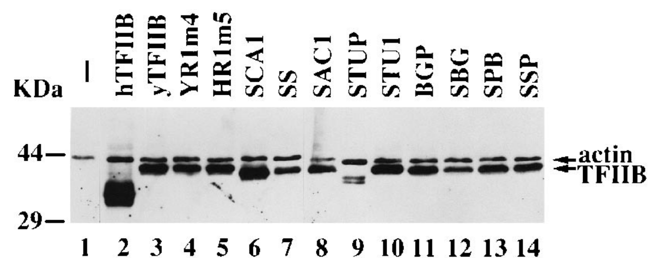

FIG. 5. Immunoblotting analysis. A human influenza virus hemagglutinin tag was fused to the indicated TFIIB proteins at their amino termini, and a monoclonal antihemagglutinin antibody was used in the immunoblotting analysis. These tagged proteins behaved similarly to the respective nontagged proteins in 5-FOA assays (data not shown). The yeast actin level, used as an internal control, was detected by a monoclonal antibody against the conserved region of actin (C4 [33]). Lane 1 represents a negative control with no tagged TFIIB protein expressed in yeast cells.

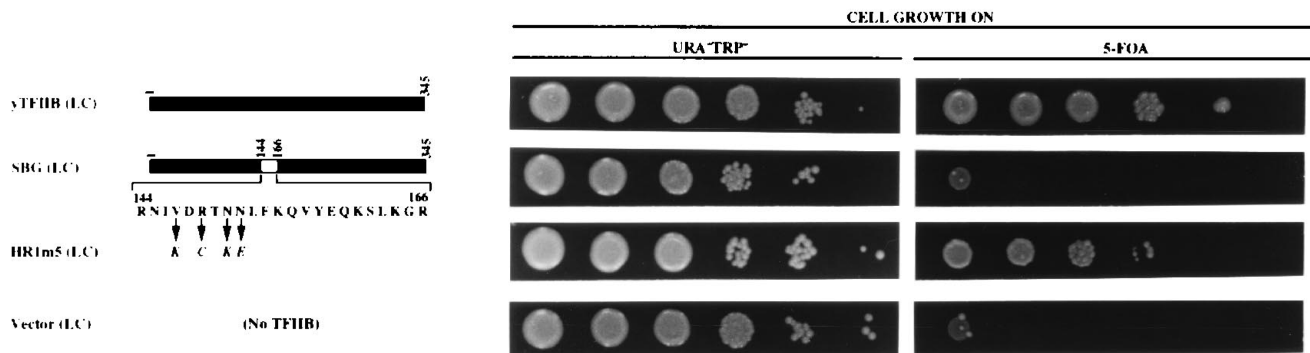

FIG. 6. TFIIB derivatives at physiological levels. The indicated TFIIB derivatives were expressed from the *yTFIIB* promoter on a low-copy-number (LC) ARS-CEN vector. Their abilities to complement the endogenous yTFIIB protein were determined in an assay similar to those for Fig. 1 and 4.

(Fig. 7). In addition, a weak start site between the two major start sites was enhanced, and several new start sites, though very weak, were detected in cells bearing the mutant yTFIIB (Fig. 7). It should be noted that despite these minor alterations, our experiments clearly demonstrate the lack of a gross shift of transcription start sites from yeast to human positions (Fig. 7 and data not shown). Therefore, it is unlikely that the defects of our mutant yTFIIB derivatives in fully supporting cell growth are solely caused by such a gross shift of transcription start sites.

Mutations in yTFIIB differentially affect gene expression in vivo. Previous studies have suggested that TFIIB plays an important role in gene regulation (see the introduction), and therefore, one potential function of the species-specific region of yTFIIB is to mediate transcriptional activation in vivo. We were interested in determining whether mutations in this region of yTFIIB would affect the ability of activators to activate transcription in yeast cells. The derivative YR1m4 enabled us to address this question because, although its in vivo activity was significantly impaired (Fig. 4), a small number of cells bearing this derivative survived 5-FOA selection. After 5-FOA selection, yeast cells bearing either YR1m4 or wild-type yTFIIB were transformed with plasmids carrying *CYC1-lacZ* reporter genes under the control of HAP1, HAP2/3/4/5, GCN4, GAL4, or no activators (15, 45). These reporter genes contain identical promoter elements from the *CYC1* gene, including the TATA boxes and transcription initiation sites, and only the activator binding sites are different.

Our reporter gene expression experiments whose results are shown in Fig. 8 demonstrate that the mutations in the species-specific region of yTFIIB can differentially affect the expression of genes activated by different activators in vivo. In these experiments the nonactivated levels of transcription in the absence of any known activators were similar in cells bearing

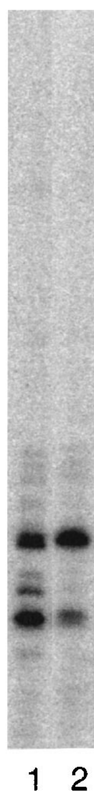

FIG. 7. Primer extension analysis. Start sites for transcription of the endogenous *ADH1* gene in cells bearing either the wild-type (lane 2) or mutant YR1m4 (lane 1) yTFIIB were determined.

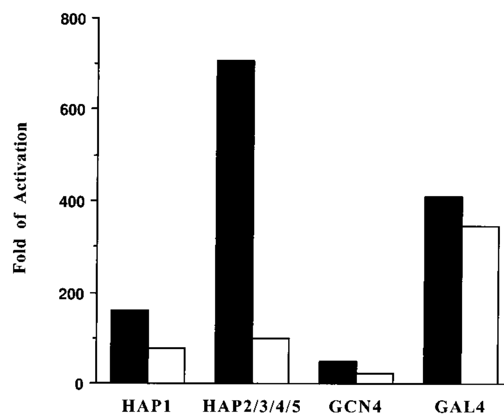

FIG. 8. Gene activation affected by mutations in the species-specific region of yTFIIB in vivo. *CYC1-lacZ* reporter genes under the control of HAP1, HAP2/3/4/5, GCN4, GAL4, or no activators were transformed into yeast cells bearing either the wild-type (solid bars) or mutant YR1m4 (open bars) yTFIIB. β-Galactosidase activities obtained from the reporter gene without any activator binding sites were used as nonactivated levels of transcription. Fold activation was calculated by dividing the β-galactosidase activities by the nonactivated levels of transcription in each strain. The binding sites for the activators were UAS1, UAS2UP1, a HIS4 oligonucleotide, and a synthetic GAL4 binding site, respectively (15, 45).

either the wild-type or the mutant yTFIIB (1.4 and 0.8 U of β-galactosidase, respectively), suggesting that the mutant yTFIIB can properly interact with other general transcription factors in vivo. Extents of activation by GAL4, as measured by fold activation over nonactivated levels of transcription, were very similar in cells bearing either the wild-type or the mutant yTFIIB (411- and 344-fold, respectively [Fig. 8]), further demonstrating that this mutant yTFIIB is not simply an inactive protein. Activation by GCN4 and HAP1 was modestly affected in cells bearing the mutant yTFIIB (Fig. 8). Consistently, we found that yeast cells bearing this mutant yTFIIB remained Gal^+ and His^+ (data not shown), two phenotypes requiring the expression of genes activated by GAL4 and GCN4, respectively. In contrast, activation by HAP2/3/4/5 was sharply decreased in cells bearing the mutant yTFIIB (from 706- to 98-fold activation). Because the mutations in the species-specific region of yTFIIB do not affect gene expression indiscriminatingly, this mutant yTFIIB (and possibly other inactive mutants) may fail to fully support cell growth because of decreased (or abolished) expression of only a subset of, rather than all, essential yeast genes.

DISCUSSION

Various general transcription factors have been shown previously to interact with transcriptional activators in vitro and suggested to respond to activators (see the introduction). Because most of these studies have been performed in vitro, one of the greatest challenges has been to determine which component(s) in the preinitiation complex receives the activation signal from transcriptional activators in living cells. Our experiments demonstrate that mutations in the species-specific region of yTFIIB can differentially affect the expression of genes activated by different activators, providing strong evidence that yTFIIB is involved in the process of transcriptional activation in vivo. Although our present experiments cannot rule out the possibility that the decreased expression of reporter genes is caused by decreased activator levels in yeast cells, such an "indirect effect" model would suggest that the mutations in the species-specific region must in turn differentially affect the expression of the activator genes.

Recent biochemical studies have identified RNA polymerase II holoenzymes from both yeast and mammalian cells (8, 28, 31, 43, 55). In addition to general transcription factors, the yeast RNA polymerase II holoenzyme also contains a class of proteins proposed to function as mediators, including GAL11 (20, 42) and SRBs (suppressors of RNA polymerase B [30, 35, 52]), as well as the SWI/SNF complex (13). The discovery of the holoenzymes suggests that the preinitiation complexes could be assembled through an alternative pathway in vivo. Irrespective of how the preinitiation complexes are assembled in vivo, any component of the preinitiation complexes could in principle be a target for transcriptional activators. It is interesting that while the mutations in the species-specific region of yTFIIB preferentially affect the expression of the target gene activated by HAP2/3/4/5, mutations in the mediator (adapter) genes *ADA2* and *ADA3* preferentially affect activators GCN4 and VP16 (4, 45). In addition, weakened GAL4 derivatives can be potentiated by a mutant GAL11 protein, suggesting a possible link between these molecules (20). Taken together, these studies suggest that different activators may transmit the activation signal to different components (or even different parts of the same component) within the preinitiation complexes in vivo.

The structure of a TBP-hTFIIB-DNA ternary complex has recently been reported (41). The helix corresponding to the species-specific region of yTFIIB is solvent exposed, and it does not contact TBP or DNA, suggesting that it could interact with other proteins. It is tempting to speculate that this helix of yTFIIB may interact with transcriptional activators, either directly or through some intermediaries. Interestingly, this helix is positioned downstream, rather than upstream, of the TATA box in the ternary complex structure (41). An interaction between yeast activators bound upstream of the TATA box and this helix of yTFIIB could potentially further stabilize the TBP-induced DNA bend (25, 27), which may be important for the assembly of the preinitiation complexes (19, 32). Alternatively, this helix could interact with other general transcription factors, in particular RNA polymerase II and/or TFIIF, on the basis of its relative location (41), an interaction that could be modulated by activators in vivo. It has been proposed that hTFIIB may undergo a conformational change upon interacting with activators (47); such a conformational change could influence how this helix of yTFIIB may interact with other general transcription factors. Further studies, involving, e.g., isolation and characterization of suppressors of the mutations in this helix of yTFIIB, would help us to further understand how the protein participates in transcriptional activation in living cells.

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