Mutational Analysis of Yeast TFIIB: A Functional Relationship Between Ssu72 and Sub1/Tsp1 Defined by Allele-Specific Interactions With TFIIB

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ABSTRACT

TFIIB is an essential component of the RNA polymerase II core transcriptional machinery. Previous studies have defined TFIIB domains required for interaction with other transcription factors and for basal transcription *in vitro*. In the study reported here we investigated the TFIIB structural requirements for transcription initiation *in vivo*. A library of *sua7* mutations encoding altered forms of yeast TFIIB was generated by error-prone polymerase chain reaction and screened for conditional growth defects. Twenty-two single amino acid replacements in TFIIB were defined and characterized. These replacements are distributed throughout the protein and occur primarily at phylogenetically conserved positions. Most replacements have little or no effect on the steady-state protein levels, implying that each affects TFIIB function rather than synthesis or stability. In contrast to the initial *sua7* mutants, all replacements, with one exception, have no effect on start site selection, indicating that specific TFIIB structural defects affect transcriptional accuracy. This collection of *sua7* alleles, including the initial *sua7* alleles, was used to investigate the allele specificity of interactions between *ssu72* and *sub1*, both of which were initially identified as either suppressors (*SUB1 2*μ) or enhancers (*sub1*Δ, *ssu72-1*) of *sua7* mutations. We show that the interactions of *ssu72-1* and *sub1*Δ with *sua7* are allele specific; that the allele specificities of *ssu72* and *sub1* overlap; and that each of the *sua7* alleles that interacts with *ssu72* and *sub1* affects the accuracy of transcription start site selection. These results demonstrate functional interactions among TFIIB, Ssu72, and Sub1 and suggest that these interactions play a role in the mechanism of start site selection by RNA polymerase II.

TRANSCRIPTION initiation by RNA polymerase II (RNAPII) requires general transcription factors (GTFs) that include the TATA-binding protein (TBP), TFIIB, TFIIF, TFIIE, and TFIIH (reviewed in Orphanides *et al.* 1996; Roeder 1996; Hampsey 1998). Although the full complement of GTFs is generally required for accurate initiation, a minimal transcription system consisting of RNAPII, TBP, TFIIB, and TFIIF is sufficient for basal initiation from certain promoters under specific conditions (Parvin and Sharp 1993; Tyree *et al.* 1993; Goodrich and Tjian 1994). *In vitro*, the GTFs assemble in a defined order on a DNA template to generate a functional transcription preinitiation complex (Buratowski *et al.* 1989; reviewed in Zawel and Reinberg 1995). These order-of-addition experiments provided the first insight into factor-factor interactions within the preinitiation complex, but do not necessarily reflect assembly of the preinitiation complex *in vivo*. This was suggested by the discovery of activation-proficient RNAPII holoenzyme complexes that include subsets of GTFs in both yeast and mammalian systems (reviewed in Koleske and Young 1995; Greenblatt 1997).

The structure of TFIIB includes a zinc-binding motif near the N terminus and two imperfect repeats in the C-terminal two-thirds of the protein (Ha *et al.* 1991; Pinto *et al.* 1992). The zinc motif and the repeats are organized into structurally distinct domains defined as a compact, protease-resistant C-terminal core (cIIB) and a protease-susceptible N-terminal region (nIIB; Barberis *et al.* 1993; Malik *et al.* 1993). cIIB is necessary and sufficient for interaction with the TBP-promoter complex, whereas nIIB interacts with RNAPII/TFIIF (Barberis *et al.* 1993; Buratowski and Zhou 1993; Ha *et al.* 1993; Hisatake *et al.* 1993; Malik *et al.* 1993; Yamashita *et al.* 1993; Bangur *et al.* 1997). The nIIB region also includes a phylogenetically conserved domain, located immediately distal to the zinc-binding domain, that affects the accuracy of initiation (Pinto *et al.* 1994).

The three-dimensional structure of cIIB has been solved by NMR and X-ray crystallography (Bagby *et al.* 1995; Nikolov *et al.* 1995). The crystal structure was determined for a cIIB-TBP-TATA ternary complex, defining specific contacts between TFIIB, TBP, and the DNA template (Nikolov *et al.* 1995). Consistent with

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footprinting (Lee and Hahn 1995) and crosslinking (Lagrange *et al.* 1996) experiments, TFIIB binds underneath and to one side of the DNA-TBP complex, contacting DNA both upstream and downstream of the TATA sequence. The TFIIB-DNA interaction appears to be sequence specific, suggesting that this interaction affects promoter strength (Lagrange *et al.* 1998).

The solution structure of unbound cIIB and the crystal structure of the cIIB-TBP-DNA complex revealed that the two imperfect repeats are arranged as two domains, each consisting of five α-helices. Although the solution and crystal structures of cTFIIB are comparable, there are several notable differences, including a more compact structure for the unbound form of cIIB and a different orientation of the two repeat domains relative to each other. Also, the A2 helix in the second repeat consists of only 5 residues in the unbound form, compared to 12 residues in the complexed form, and a short helix (designated F2) located at the end of the second repeat is unique to the complexed form (Hayashi *et al.* 1998). No three-dimensional structure is available for full-length TFIIB, although an NMR structure for the N-terminal region of TFIIB from the archaebacterium *Pyrococcus furiosus* revealed that the metal-binding domain forms a zinc ribbon (Zhu *et al.* 1996).

This structural information implies that TFIIB is pliable. Indeed, VP16 induces a conformational change in TFIIB (Roberts and Green 1994), and binding of either nIIB or the activation domain of VP16 causes free cIIB to adopt a conformation similar to that of cIIB in the DNA-bound form (Hayashi *et al.* 1998). This conformational change is likely to be physiologically significant since the Pho4 transcriptional activator induces a similar change in yeast TFIIB, and a TFIIB derivative that blocks the Pho4-TFIIB interaction fails to express the *PHO5* gene in an activator-specific manner (Wu and Hampsey 1999).

Yeast TFIIB is encoded by the *SUA7* gene, which was first identified on the basis of the ability of *sua7* mutations to alter the accuracy of transcription initiation (Pinto *et al.* 1992). The *sua7-1* and *sua7-3* alleles encode glutamate-62→lysine (E62K) and arginine-78→cysteine (R78C) replacements, respectively, and cause dramatic downstream shifts in start site selection at the *CYC1* and *ADH1* genes (Pinto *et al.* 1994). Recessive suppressors of the *sua7-1* mutation identified the genes encoding the largest subunit of TFIIF (*ssu71*/*TFG1*; Sun and Hampsey 1995) and the Rpb9 (*ssu73*/*RPB9*) subunit of RNAPII (Sun *et al.* 1996). These mutations not only suppressed the *sua7-1* cold-sensitive growth defect, but also partially or completely restored the normal initiation pattern.

An enhancer of the *sua7-1* mutation has also been identified. The *SSU72* gene was identified on the basis of the synthetic heat-sensitive phenotype of an *sua7-1 ssu72-1* double mutant (Sun and Hampsey 1996b). Moreover, the *sua7-1*-mediated downstream start site shift at *ADH1* was dramatically enhanced in the double mutant, an effect that is dependent upon both alleles. Another enhancer of *sua7* mutations, also encoding amino acid replacements at E62 and R78, has also been identified. In this case, the *SUB1* gene was initially identified as a high-copy suppressor of E62G and R78H replacements; deletion of the *SUB1* gene was subsequently shown to be lethal in combination with E62G and R78H (Knaus *et al.* 1996). *SSU72* is an essential gene encoding a novel protein of unknown function that is conserved among eukaryotic organisms (Sun and Hampsey 1996a). The Sub1 protein is identical to yeast Tsp1, identified biochemically on the basis of its ability to stimulate basal transcription (Henry *et al.* 1996), and is homologous to the human transcriptional coactivator PC4 (Ge and Roeder 1994; Kretzschmar *et al.* 1994).

In an effort to identify amino acid residues within TFIIB that are critical for specific functions *in vivo*, we have generated and characterized a collection of random *sua7* mutants that encode single amino acid replacements within TFIIB. These mutants were characterized with respect to protein stability, effects on start site selection, and genetic interactions with Ssu72 and Sub1. Our results demonstrate that only specific TFIIB replacements alter the accuracy of start site selection. Furthermore, *sub1*Δ and *ssu72-1* are allele specific with respect to *sua7* interactions and the specificity of these two sets of interactions overlaps. These results functionally link Ssu72 and Sub1 and implicate both proteins in the mechanism of start site selection.

MATERIALS AND METHODS

Strains, media, and nomenclature: The *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Unless otherwise indicated, all media were prepared according to standard recipes (Sherman 1991). 5-FOA medium contains 5-fluoroorotic acid and was used as a positive selection against the *URA3*$^+$ gene (Boeke *et al.* 1984). The heat- (Tsm$^-$) and cold-sensitive (Csm$^-$) phenotypes denote impaired growth relative to the isogenic *SUA7*$^+$ strain YMH130 at 37° and 16°, respectively. These phenotypes were initially identified on rich (YPD) medium, but were subsequently scored on synthetic complete (SC) medium, which diminished the inherent cold sensitivity associated with the *trp1* marker on YPD medium.

Yeast and *E. coli* transformations: Yeast transformations were done by the lithium acetate procedure (Gietz *et al.* 1992). Plasmid DNA was recovered from yeast as described previously (Hoffman and Winston 1987) and introduced into *Escherichia coli* by electroporation (Dower *et al.* 1988). *E. coli* strain XL1-Blue (*recA1*, *endA1*, *gyrA96*, *thi-1*, *supE44*, *hsdR17* (r_k^- m_k^-) *relA1 lac*, [*F′ proAB lacI*^{Q} *lacZ*Δ*M15 Tn10* (*tet′*)]) was used as the host strain for all plasmids.

Error-prone PCR mutagenesis of *SUA7*: Error-prone PCR (EP-PCR) was performed according to the procedures described previously (Muhlrad *et al.* 1992). The reaction mixture contained the following components: (i) 10 ng pM299 (*SUA7*) template DNA; (ii) 10× PCR buffer II (Perkin-Elmer, Norwalk, CT); (iii) 0.2 mm of one "biased" dNTP; (iv) 1 mm of the three other dNTPs; (v) 3 mm $MgCl_2$ and 0.2 mm $MnCl_2$; (vi) 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer);

TABLE 1

Yeast strains used in this study

Strain[a]	Genotype
YIP91-13B	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pDW11:*SUA7 URA3 CEN*]
YMH97	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 [pDW11:SUA7-URA3*]
YMH130	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM299: *SUA7 HIS3 CEN*]
YMH131	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM326: *sua7-1 HIS3 CEN*]
YMH132	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM325: *sua7-3 HIS3 CEN*]
YMH134	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM342: *sua7-5 HIS3 CEN*]
YMH137	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM365: *sua7-8 HIS3 CEN*]
YMH140	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM376: *sua7-11 HIS3 CEN*]
YMH141	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM377: *sua7-12 HIS3 CEN*]
YMH142	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM378: *sua7-13 HIS3 CEN*]
YMH148	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM391: *sua7-17 HIS3 CEN*]
YMH149	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM392: *sua7-18 HIS3 CEN*]
YMH151	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM395: *sua7-19 HIS3 CEN*]
YMH175	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM415: *sua7-20 HIS3 CEN*]
YMH285	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM416: *sua7-21 HIS3 CEN*]
YMH287	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM400: *sua7-23 HIS3 CEN*]
YMH288	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM401: *sua7-24 HIS3 CEN*]
YMH290	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM402: *sua7-26 HIS3 CEN*]
YMH292	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM404: *sua7-28 HIS3 CEN*]
YMH293	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM500: *sua7-29 HIS3 CEN*]
YMH295	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM502: *sua7-31 HIS3 CEN*]
YMH297	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM504: *sua7-33 HIS3 CEN*]
YMH298	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM505: *sua7-34 HIS3 CEN*]
YMH299	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM506: *sua7-35 HIS3 CEN*]
YMH300	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM507: *sua7-36 HIS3 CEN*]
YMH304	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM511: *sua7-40 HIS3 CEN*]
YMH305	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM512: *sua7-41 HIS3 CEN*]
YMH306	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM513: *sua7-42 HIS3 CEN*]
YMH307	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM514: *sua7-43 HIS3 CEN*]
YMH308	*MAT***a** *his3-Δ1 leu2-3, 112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM515: *sua7-44 HIS3 CEN*]
YMH422	*MAT***a** *his3-Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 sua7::LEU2* [pDW11:*SUA7 URA3 CEN*]
FY833	*MAT***a** *his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63*
YMH476	*MAT***a** *his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 sub1Δ::hisG*
YMH477	*MAT***a** *his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 sua7::LEU2 sub1Δ::hisG* [pDW11: *SUA7 URA3 CEN*]
YDP248	*MAT***a** *ura3-52 leu2-3, 112 trp1Δ::ura3 sua7-1 ssu72-1*
YMH504	*MAT***a** *ura3-52 leu2-3, 112 trp1Δ::ura3 sua7-1 ssu72-1 his3Δ::ura3*
YMH505	*MAT***a** *his3Δ::ura3 ura3-52 leu2-3, 112 trp1Δ::ura3 sua7::LEU2 ssu72-1* [pDW11: *SUA7 URA3 CEN*]

[a] YIP91-13B and YMH130 were described previously (Pinto *et al.* 1994). FY833 was from F. Winston (Winston *et al.* 1995). Strains YMH131-YMH308 are isogenic to YMH130 and were derived by plasmid shuffle. The *sua7* plasmid shuffle derivatives of YMH477 (*sub1*Δ) and YMH505 (*ssu72-1*) are not listed, but are available upon request.

(vii) 1 μm of primers oIP-81 [5′-TTTCTAGACGCTGGTGTG GACGATC (*SUA7* coding strand, position −54 to −34, plus *Xba*I overhang)] and oIP-82 [5′-CGAATTCCCGTGCTTCTT GTTCCTA (*SUA7* template strand, position 1086 to 1062, plus *Eco*RI overhang)]. The volume of the reaction mixture was 100 μl. Amplification conditions were 33 cycles of 94° for 1 min, 45° for 2 min, and 72° for 3 min. Four reactions, each biased against one dNTP, were pooled following amplification.

Plasmid shuffle: The pooled EP-PCR reaction mixes were combined with pM362 (*HIS3 sua7 CEN*) that had been gapped within *sua7* by digestion with *Bsp*EI and *Bam*HI. This mixture was introduced into strain YIP91-13B, selecting for transformants on −His medium. Strain YIP91-13B contains a disruption of the chromosomal *SUA7* locus (*sua7::LEU2*); cell viability is maintained by plasmid pDW11 (*SUA7 URA3 CEN*; Pinto *et al.* 1992). The gapped plasmid includes sequences identical to both ends of the amplified DNA, allowing for the gap to be repaired *in vivo* by homologous recombination (Muhlrad *et al.* 1992). Master plates representing ~4000 His^+ transformants were made. The *SUA7* wild-type gene, carried on a *URA3* vector, was then counterselected on 5-FOA medium, allowing for phenotypes associated with plasmid-borne *sua7* alleles to be scored in the absence of wild-type *SUA7*. The *sua7* allele in pM362 carries a nonsense mutation at codon 62 (Trp → stop), which eliminates any *SUA7* wild-type background. Accordingly, the YMH series of strains are isogenic derivatives of strain YIP91-13B, differing from each other only by the *sua7* allele carried on derivatives of the *HIS3* plasmid pM362.

A second plasmid shuffle system was constructed in isogenic *SUB1* wild-type (FY833) and *sub1*Δ (YMH476) strains. YMH476 was constructed by transforming the *Pvu*II–*Eco*RI fragment encompassing the *sub1Δ::hisG-URA3-hisG* cassette into FY833, selecting for Ura^+ transformants, and subsequently selecting for FOA^r colonies to recover the *ura3* marker. YMH476 was transformed with pDW11 [*SUA7 URA3 CEN*] and the chromo-

somal *SUA7* gene was subsequently disrupted (*sua7::LEU2*) as described previously (Pinto *et al.* 1992). The resulting strain, YMH477, was used as the host to shuffle *sua7* alleles into the *sub1*Δ background, as described above for YIP91-13B.

A third plasmid shuffle system was set up using strain YDP248 (*ssu72-1 sua7-1*). The *HIS3* gene of YDP248 was disrupted (*his3*Δ*::URA3*) and the *ura3* marker was recovered by selection for spontaneous FOA resistance, yielding strain YMH504. YDP504 was transformed with plasmid pM299 (*SUA7 HIS3 CEN*) and the chromosomal *sua7-1* allele was subsequently disrupted (*sua7::LEU2*; Pinto *et al.* 1992). The resulting strain, YMH505, was used as the host to shuffle *sua7* alleles into the *ssu72-1* background, as described above.

DNA sequence analysis: Plasmid DNA was recovered from each of the YMH strains and introduced into *E. coli* strain XL1-Blue by electroporation. Single-stranded DNA was isolated using the VCS M13 helper bacteriophage (Stratagene, La Jolla, CA) in the presence of kanamycin. The *sua7* coding region was sequenced in its entirety for each allele by the dideoxy-terminator method using three *SUA7* template strand primers (oIP-82, 1086 → 1062; oIP-133, 676 → 698; and oIP-125, 314 → 298; ATG start codon is designated +1).

Western blot analysis: Yeast strains were grown in YPD medium to A_{600} = 1.0. Cells were harvested, washed, and total protein was extracted into electrophoresis sample buffer. Western blotting was performed according to standard procedures using 10% polyacrylamide gels. The primary antibody was rabbit anti-TFIIB (Pinto *et al.* 1994); rabbit anti-Leu4 was used as a loading control. Antigen-antibody complexes were detected by chemiluminescence using goat anti-rabbit IgG conjugated to horseradish peroxidase. The TFIIB signal was quantified using the public domain NIH Image program (http://rsb.info.nih.gov/nih-image/) and normalized to the Leu4 signal. Accordingly, the TFIIB/Leu4 ratio was defined as 1.0 for the wild type in each panel.

Determination of transcription start sites: Primer extension was performed as described previously, using total RNA and the *ADH1*-specific primer oIP-87 (Pinto *et al.* 1992). Primer extension products were resolved in a 6% polyacrylamide DNA sequencing gel and visualized by autoradiography. The molecular size markers correspond to *SUA7* DNA sequenced with primer oIP-133 (Pinto *et al.* 1992).

GST pull-down experiments: A total of 3 μg of glutathione *S*-transferase (GST), GST-TFIIB, GST-E62K, or GST-R78C was incubated with 20 μl bed volume of glutathione-Sepharose beads in 300 μl of Buffer T (10 mm tris-acetate, pH 7.9; 100 mm NaCl; 10% glycerol; 1 mm EDTA; 1 mm DTT) containing 0.01% NP40 at 4° for 1 hr. Input Ssu72 protein was generated by *in vitro* transcription/translation using the TNT kit (Promega, Madison, WI). A total of 5 μl of input protein was incubated with GST and its derivatives at 4° for 4 hr. Beads were collected by centrifugation, washed three times with Buffer T containing 300 mm NaCl and 0.05% NP40, and boiled in 15 μl loading buffer. Proteins were resolved by SDS-PAGE and visualized by autoradiography.

RESULTS

Isolation of *sua7* mutants: A library of *sua7* mutants was generated by EP-PCR as described in the materials and methods. Following plasmid shuffle, FOA-resistant colonies were spotted onto three sets of rich (YPD) medium and incubated at normal (30°), reduced (16°), and elevated (37°) temperatures. Strains that failed to grow, or grew very poorly at either 16° or 37° relative to growth of the same strain at 30°, were defined as cold sensitive (Csm^-) or heat sensitive (Tsm^-), respectively. To confirm that phenotypes were conferred by plasmid-borne *sua7* alleles, plasmid DNA was extracted from each strain and reintroduced into strain YIP91-13B, and FOA-resistant colonies were rescored at 16° and 37°. Strains were then crossed with strain YMH97 ($SUA7^+$) and the resulting diploid strains were scored at 16° and 37° to assess dominance/recessiveness of each *sua7* allele. Most *sua7* mutations were recessive, although five exhibited semidominance with respect to growth defects at either 16° or 37°. Results are summarized in Table 2.

Characterization of *sua7* mutants: Plasmid DNAs from 33 Csm^- and/or Tsm^- mutants were recovered and the DNA sequence of the entire *SUA7* open reading frame was determined for each mutant. One or more base pair substitutions within the *SUA7* coding region were identified for all 33 *sua7* alleles. Twenty-two of the 33 alleles encode single amino acid replacements at 21 different positions. The remaining 11 alleles encode two or more replacements. Only the mutants encoding single residue replacements are described here. These replacements are summarized in Table 2, which also includes the E62K, E62R, and R78C single mutants and E62K-R78C and E62R-R78E double replacements, which were defined previously (Pinto *et al.* 1994).

The collection of single amino acid replacements in TFIIB are distributed throughout the length of the protein (Figure 1). Comparison with the phylogenetic series of TFIIB sequences revealed that most replacements occur at conserved positions; only the N19D, P25L, C149Y, and H158R replacements occur at variable positions (Na and Hampsey 1993). Replacements were identified in each of the structurally defined domains of TFIIB, including the zinc ribbon, both repeats of the core domain, and the phylogenetically conserved region between the zinc ribbon and the core. However, replacements within the zinc ribbon and in the first two helices of the second repeat of the core domain (A2 and B2) were underrepresented. This distribution underscores the functional importance of the entire TFIIB sequence and suggests that the zinc ribbon and part of the core domain are especially intolerant of amino acid replacements.

Western blot analysis was used to determine the effects of each amino acid replacement on the steady-state level of TFIIB (Figure 2). Many replacements did not adversely affect TFIIB levels and some even appeared to enhance TFIIB stability, although in no case was the increase greater than twofold. Other replacements reproducibly diminished TFIIB levels. Among the six amino acid replacements near the C terminus, only K3101 had no appreciable effect on stability and L284Q and F289S diminished steady-state levels by at least fivefold. The only other replacements to cause a significant decrease in TFIIB levels were P25L, which lies within the zinc ribbon, and H158R, a component of the B1 helix of the first repeat. Thus, many of the amino acid

TABLE 2

Summary of TFIIB amino acid replacements, their phenotypes, and interactions with Sub1 and Ssu72

Strain	yTFIIB amino acid replacement[a]	hTFIIB amino acid[b]	D/r[c]	Phenotypes[d]	Effect[e] on start site selection	Effect[f] of [*SUB1 2μ*] *sub1Δ*		Effect[g] of [*SSU72 2μ*] *ssu72-1*	
YMH308	NI9D	P11	r	Csm$^-$	—	—	—	—	—
YMH175	P25L	P16	SD	Csm$^-$	—	—	—	—	—
YMH304	V51A	V40	SD	Csm$^\pm$ Tsm$^-$	—	—	—	—	—
YMH298	L52P[i]	V41	SD	Csm$^-$ Tsm$^-$	—	—	—	—	—
YMH300	S53P[i]	G42	r	Csm$^-$ Tsm$^-$	—	—	—	—	Csm^{--}
YMH285	L56P	V45	r	Csm$^-$	—	—	—	—	—
YMH131[h]	E62K[i]	E51	r	Csm$^-$ Tsm$^\pm$	Strong shift	Csm$^+$	Dead	Tsm$^+$	Csm^0
YMH137[h]	E62R[i]	E51	r	Csm$^-$ Tsm$^\pm$	Strong shift	Csm$^+$	Dead	Tsm$^+$	Csm^0
YMH134[h]	E62K-R78C	E51-R66	r	Csm$^-$ Tsm$^-$	Strong shift	Csm$^+$	Dead	Tsm$^+$	Csm^0
YMH151[h]	E62R-R78E	E51-R66	r	Csm$^-$ Tsm$^-$	Strong shift	Csm$^+$	Dead	Tsm$^+$	Csm^0
YMH132[h]	R78C[i]	R66	r	Csm$^-$ Tsm$^\pm$	Strong shift	Csm$^+$	Dead	Tsm$^+$	Csm^0
YMH292	T101P	G89	r	Csm$^-$	—	—	—	ND	ND
YMH140	L136P	M124	SD	Csm$^\pm$ Tsm$^-$	—	—	—	—	—
YMH141	V146M	I134	r	Csm$^-$ Tsm$^\pm$	—	—	—	—	—
YMH142	C149Y[i]	R137	r	Csm$^-$ Tsm$^\pm$	Weak shift	Csm$^+$	Sick	Tsm$^+$	Slg$^-$
YMH288	H158R	Y146	r	Csm$^-$	—	—	—	—	—
YMH297	K166E	R154	r	Tsm$^-$	—	—	—	—	—
YMH299	K166Q	R154	r	Tsm$^-$	—	—	—	—	—
YMH293	K201I	K189	r	Csm$^-$ Tsm$^-$	—	—	—	—	—
YMH290	N208S	K196	r	Csm$^-$	—	—	—	—	—
YMH306	L214S	L202	SD	Csm$^-$	—	—	—	—	—
YMH307	S273A	S249	r	Csm$^-$	—	—	—	—	—
YMH295	L284Q	M260	r	Csm$^\pm$ Tsm$^-$	—	—	—	—	—
YMH148	F289S	S265	r	Csm$^\pm$ Tsm$^-$	—	—	—	—	—
YMH305	K310I	R286	r	Csm$^-$	—	—	—	ND	ND
YMH287	L316P	I292	r	Csm$^-$	—	—	—	ND	ND
YMH149	L323P	L299	r	Csm$^\pm$ Tsm$^-$	—	—	—	Tsm$^+$	—

[a] The yeast TFIIB (yTFIIB) numbering system and single-letter amino acid designations are used.

[b] Denotes corresponding amino acids in human TFIIB (hTFIIB).

[c] Dominance/recessiveness of the indicated *sua7* alleles was assessed by crossing each strain with YMH97 and the resulting diploid was scored for growth at 16° and/or 37°. SD, semidominant; r, recessive.

[d] Csm$^-$ and Tsm$^-$ denote dramatically impaired growth on YPD medium at 16° and 37°, respectively, relative to the isogenic *SUA7* strain (YMH130) under the same conditions. Csm$^\pm$ and Tsm$^\pm$ denote impaired growth, although to a lesser extent than Csm$^-$ and Tsm$^-$.

[e] Effects on start site selection were determined at the *ADH1* gene. "Strong shift" denotes diminished initiation at position −37 and enhanced initiation at sites downstream of −27. "Weak shift" denotes a subtle, but reproducible, shift resulting in enhanced initiation at −30. These data are summarized from Figure 4.

[f] Csm$^+$ denotes suppression of the Csm$^-$ phenotype on YPD medium at 16°. "Dead" denotes failure of the plasmid shuffle strain (*sua7::LEU2*) carrying plasmid-borne *SUA7 URA3* and *sua7 HIS3* to grow on FOA medium, indicating that the *sua7* allele encoding the indicated amino acid replacement is lethal in combination with *sub1Δ*.

[g] Csm^0 denotes almost no growth at 16° and is depicted in Figure 5. The growth phenotype of the S53P *ssu72-1* strain is more pronounced than the Csm$^-$ phenotype of the S53P *SSU72* strain and is therefore designated Csm^{--}. ND, potential interactions between *SSU72 2μ* or *ssu72-1* and the *sua7* alleles encoding T101P, L284Q, and L316P were not determined.

[h] Strains YMH131, YMH132, YMH134, YMH137, and YMH151 were defined previously (Pinto *et al.* 1994).

[i] Amino acid replacements at these positions were also identified in a screen for heat- or cold-sensitive *sua7* mutants by Guarente and co-workers (Knaus *et al.* 1996) or by site-directed mutagenesis (Shaw *et al.* 1996; Bangur *et al.* 1997, Pardee *et al.* 1998). The L52P replacement has been identified in two independent studies (Knaus *et al.* 1996; Pardee *et al.* 1998); L52P is lethal in the strain background described by Ponticelli and co-workers. A C149R replacement is phenotypically Tsm$^-$ (Knaus *et al.* 1996); C149R was also described by Ma and co-workers as one of four TFIIB amino acid replacements encoded by the YR1m4 mutant (Shaw *et al.* 1996).

replacements described here appear to affect TFIIB function, although specific replacements in defined regions of the protein appear to affect stability, implying that the growth defects associated with these mutants are due to diminished levels of TFIIB.

Effects of TFIIB derivatives on start site selection *in vivo*: All of the TFIIB derivatives described here were assayed for potential effects on start site selection at the *ADH1* gene by primer extension (Figure 3 and data not shown). The *ADH1* gene was chosen for this analysis

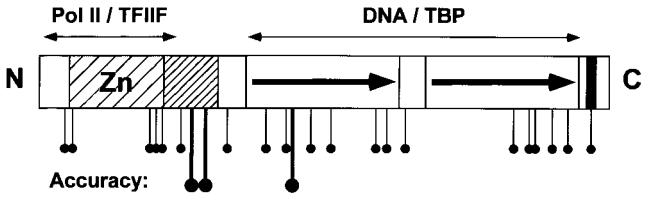

Figure 1.—Schematic summary of TFIIB amino acid replacements. The single amino acid replacements generated by EP-PCR are denoted by small ball-and-stick figures at their approximate positions within TFIIB and are summarized in Table 2. Large ball-and-stick figures denote positions of amino acid replacement that affect the accuracy of start site selection, which, with the exception of the C149Y replacement within the first repeat of the core domain, arose in the *sua* genetic selection for genes that affect start site selection (Pinto *et al.* 1994). Zn denotes the zinc ribbon domain; the narrow crosshatch denotes the phylogenetically conserved domain immediately downstream of the zinc ribbon; the two arrows denote the two imperfect repeats of the core domain; and the black rectangle near the C terminus denotes the small helical structure that is specific to the bound form of TFIIB (Hayashi *et al.* 1998). The N-terminal domain interacts directly with RNAPII and TFIIF and the core domain interacts with TBP and the DNA template (Ha *et al.* 1993).

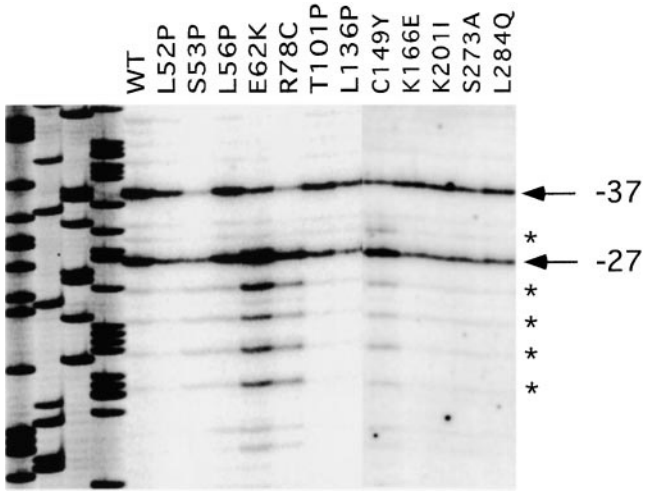

Figure 3.—Effects of TFIIB amino acid replacements on the accuracy of transcription initiation at the *ADH1* gene. Start sites were mapped by primer extension analysis. The TFIIB amino acid replacements are denoted by the single letter code at the top of each lane. The downstream shift caused by the E62K and R78C replacements was reported previously (Pinto *et al.* 1994). The initiation pattern associated with the C149Y replacement is different from the initiation pattern associated with E62K and R78C, but similar to the effect of a quadruple replacement that includes a C149R replacement (Shaw *et al.* 1996). All other replacements had little or no effect on start site selection.

because specific amino acid replacements in TFIIB (*sua7*) and the Rpb1 (*sua8*) subunit of RNAPII were shown previously to affect the accuracy of initiation at *ADH1*, yet initiation at several other genes was unaffected (Berroteran *et al.* 1994; Pinto *et al.* 1994). Transcription at *ADH1* normally initiates with equal frequency at positions −37 and −27 relative to the ATG start codon (lane 1, WT). Hallmarks of start site effects at *ADH1* are diminished initiation at position −37 relative to −27 and enhanced initiation at sites downstream of −27 (Pinto *et al.* 1992, 1994; Sun and Hampsey 1996b). Consistent with previous results, E62K and R78C shifted initiation downstream of normal. However, with the exception of the C149Y replacement, none of the other replacements significantly affected start site selection at *ADH1*. The C149Y replacement diminished initiation at −37 relative to −27 and enhanced initiation at −30 (Figure 3). A similar effect on start sites at *ADH1* was also observed in a *sua7* mutant that encodes four single amino acid replacements, including C149R (Shaw *et al.* 1996). These results demonstrate that the first repeat of core TFIIB is also involved in the accuracy of initiation, yet most TFIIB replacements do not alter initiation. Thus, specific TFIIB defects, rather than general structural abnormalities, account for altered start site selection.

Allele specificity of *sua7* interactions with *sub1*: The yeast *SUB1* gene was identified as a high-copy suppressor of R78H and E62G replacements in TFIIB (Knaus *et al.* 1996). The extensive collection of *sua7* mutants described here presented an opportunity to further investi-

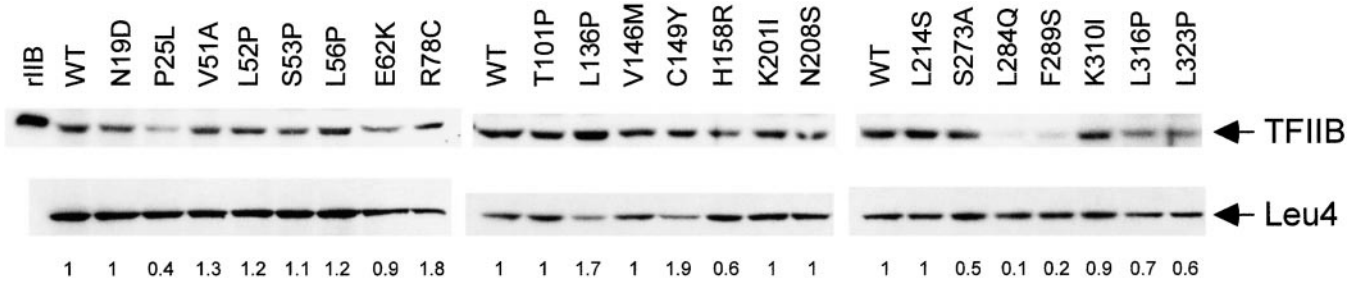

Figure 2.—Western blot analysis of TFIIB derivatives. Crude cell extracts were prepared and analyzed as described in materials and methods. Leu4 was used as a loading control. rIIB denotes recombinant TFIIB. The TFIIB amino acid replacements are denoted by the single letter code at the top of each lane. These strains are isogenic, differing only by the plasmid-encoded *sua7* allele. The TFIIB signal was quantified and normalized to the Leu4 signal. Accordingly, the TFIIB/Leu4 ratio is defined as 1.0 for the wild-type (WT) strain in each panel.

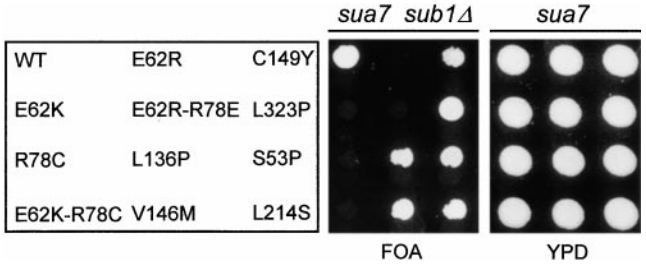

Figure 4.—Allele specificity of *sua7 sub1*Δ interactions. Strain YMH477 (*sua7::LEU2 sub1*Δ [*SUA7 CEN URA3*]) and the isogenic strain YMH422 (*sua7::LEU2 SUB1*) were transformed with *sua7 HIS3 CEN* plasmid DNA encoding either wild-type TFIIB or the indicated amino acid replacements. His^+ transformants were selected, subcloned, and replica printed onto 5-FOA medium to counterselect the wild-type *SUA7* plasmid. No 5-FOA-resistant colonies arose for *sub1*Δ in combination with *sua7* alleles encoding amino acid replacements at positions E62 or R78, yet these same alleles resulted in 5-FOA-resistance in the *SUB1*$^+$ background. Thus, E62 and R78 replacements are lethal in combination with *sub1*Δ, whereas all other TFIIB replacements are viable. Although not lethal, *sua7-13* (C149Y) exhibited impaired growth in combination with *sub1*Δ. Data are summarized for all *sua7* alleles in Table 2.

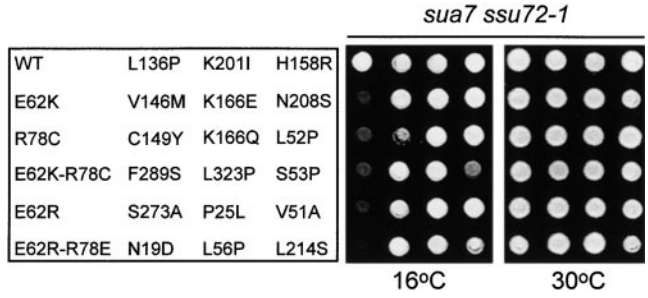

Figure 5.—Allele specificity of *sua7 ssu72-1* interactions. Strain YMH505 (*sua7::LEU2 ssu72-1* [*SUA7 CEN URA3*]) was transformed with *sua7 HIS3 CEN* plasmids encoding either wild-type TFIIB or the indicated amino acid replacements. His^+ transformants were selected and subsequently replica printed onto 5-FOA medium to counterselect the *SUA7*$^+$ plasmid. Cells were then spotted onto YPD medium and grown at either 16° (6 days) or 30° (2 days). *The ssu72-1* mutation enhanced the Csm^- phenotypes associated with the *sua7* alleles encoding either single or double amino acid replacements at positions E62 and R78 and, to a lesser extent, the S53P and C149Y replacements. These enhanced Csm^- phenotypes can be rescued by plasmid-borne, wild-type *SSU72*, confirming that the synthetic phenotypes are a consequence of the specific *sua7* mutations in combination with *ssu72-1*. These data are summarized in Table 2.

gate the interaction between TFIIB and Sub1. Accordingly, we scored for potential suppression of *sua7* Csm^- phenotypes by high-copy *SUB1* expression and synthetic enhancement of *sua7* phenotypes in combination with a *sub1*Δ deletion. Similar to its effects on E62G and R78H, high-copy *SUB1* suppressed the phenotypes associated with E62K, E62R, and R78C single replacements and the E62K-R78C and E62R-R78E double replacements (Table 2). Interestingly, the Csm^- growth defect of the C149Y replacement within helix B1 was also suppressed by high-copy *SUB1*. Thus, suppression by high-copy *SUB1* correlates with *sua7* alleles that affect start site selection (summarized in Table 2).

Disruption of the nonessential *SUB1* gene (*sub1*Δ) caused synthetic lethality in combination with E62G and R78H (Knaus *et al.* 1996). Similarly, we found that *sub1*Δ was lethal in combination with all E62 and R78 replacements, including E62K-R78C and E62R-R78E double replacements (Figure 4; summarized in Table 2). The *sub1*Δ deletion also enhanced the growth defect of the C149Y replacement, resulting in a slow growth phenotype comparable to that described previously for a C149R *sub1*Δ mutant (Knaus *et al.* 1996), although this effect was much less pronounced than the lethal effect of *sub1*Δ in combination with E62 and R78 replacements. Thus, high-copy *SUB1* and *sub1*Δ exert opposite effects on the growth phenotypes associated with all TFIIB mutations that alter start site selection, yet have no discernable effect on TFIIB replacements that do not affect the accuracy of initiation.

Allele specificity of *sua7* interactions with *ssu72-1*: The *ssu72-1* allele was identified in a genetic screen for suppressors of the Csm^- phenotype associated with *sua7-1* (E62K). However, in the original suppressor strain (YZS19), two genes were identified that interacted with *sua7-1: ssu73-1/rpb9* suppressed the *sua7-1* Csm^- phenotype, whereas *ssu72-1* enhanced the *sua7-1* defect, resulting in a Tsm^- phenotype (Sun and Hampsey 1996a). Furthermore, the *sua7-1 ssu72-1* double mutation dramatically shifted start site selection at *ADH1* downstream of normal (Sun and Hampsey 1996a). In an effort to further define the relationship between TFIIB and Ssu72, we used our collection of *sua7* alleles to determine if *ssu72-1* is allele specific with respect to enhancement of *sua7* mutations. As described above for *sub1*Δ, this was done by plasmid shuffle in an *ssu72-1* background, using the *sua7* alleles described in Table 2. Although many of the *sua7* alleles impaired growth at 16° (Table 2), *ssu72-1* dramatically enhanced this phenotype in an allele-specific manner. Accordingly, *ssu72-1* essentially eliminated growth in *sua7* strains encoding the E62K, E62R, and R78C single residue replacements and the E62K-R78C and E62R-R78E double replacements (Figure 5; summarized in Table 2). The only other *sua7* alleles affected by *ssu72-1* encode S53P and C149Y, also resulting in enhanced Csm^- growth defects, albeit to a lesser extent than the alleles encoding E62 and R78 replacements. Furthermore, high-copy expression of *SSU72*$^+$ from a 2μ plasmid suppressed the weak Tsm^- phenotypes of *sua7* mutants encoding E62, R78, and C149 replacements, yet, with the exception of L323P, did not affect the Tsm^- phenotype of other *sua7* mutants at 37° (Table 2). Although *ssu72-1* did not enhance the Csm^- phenotype of L323P, it did confer a synthetic slow-growth phenotype at 30° (Table 2). Thus, *ssu72-1* and high-copy *SSU72* display allele specificity

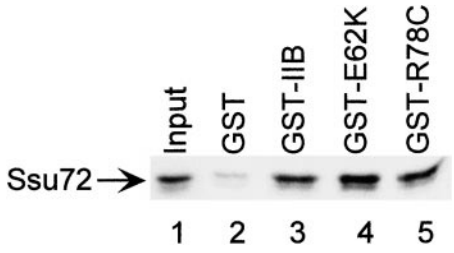

Figure 6.—Direct interaction between TFIIB and Ssu72. Equal amounts of GST alone or GST fused to full-length TFIIB or its E62K or R78C derivatives were incubated with ^{35}S-labeled Ssu72 protein (input). Following centrifugation of immobilized GST, samples were washed with buffer containing 300 mm NaCl, 0.05% NP-40, denatured in sample buffer, fractionated by SDS-PAGE, and analyzed by autoradiography.

with respect to enhancement and suppression of *sua7* defects and this specificity correlates (with the exceptions of S53P and L323P) with TFIIB derivatives that affect the accuracy of initiation. Moreover, this specificity overlaps the allele specificity of the *sua7 sub1*Δ interactions.

TFIIB interacts directly with Ssu72: The allele specificity of the *sua7 ssu72* interaction indicates a specific functional relationship, possibly involving direct contact, between TFIIB and Ssu72. We determined whether Ssu72 directly interacts with TFIIB by GST pull-down assays. Input protein for these assays was generated by *in vitro* transcription and translation of Ssu72. Equal amounts of GST protein and its TFIIB derivatives were used in these assays, as determined by Coomassie stained SDS/PAGE-resolved protein samples (data not shown). Results are shown in Figure 6. Incubation of GST-TFIIB with ^{35}S-labeled Ssu72 resulted in recovery of the Ssu72 protein (lane 3) that was substantially greater than with the GST control (lane 2). Neither the E62K nor R78C replacement significantly weakened the Ssu72-TFIIB interaction (lanes 4 and 5). Thus, Ssu72 directly binds TFIIB *in vitro*, a result consistent with the allele specificity of *sua7 ssu72-1* interactions *in vivo*. However, the enhanced Csm$^-$ phenotypes associated with the E62K and R78C replacements do not appear to be a consequence of weakened Ssu72-TFIIB interactions.

DISCUSSION

This article defines a collection of 22 single amino acid replacements in yeast TFIIB. Each of these *sua7* mutants was generated by EP-PCR and selected solely on the basis of heat- and/or cold-sensitive growth defects. Accordingly, these mutants were isolated without regard for effects on any specific function associated with TFIIB, including DNA-TBP-TFIIB ternary complex formation, TFIIB-RNAPII interaction, effects on either the rate or accuracy of transcription initiation, or interaction with gene-specific activator proteins. The amino acid replacements are distributed throughout the length of TFIIB, although the zinc ribbon and part of the second repeat of the core domain appear to be hypomutable or mutations in these regions do not confer conditional growth defects. Two of the replacements reported here were also found in other studies. L52P and S53P were also generated by EP-PCR and identified as Csm$^-$ mutants (Knaus *et al.* 1996), although L52P has also been reported to be an inviable replacement (Pardee *et al.* 1998).

The role of TFIIB in start site selection: The *SUA7* gene was initially identified in a genetic selection for mutants that shifted transcription start site selection downstream of normal (Pinto *et al.* 1992). Three mutants encoding either E62K or R78C replacements were defined (Pinto *et al.* 1994). On the basis of the effects of single- and double-residue replacements, it was suggested that E62 and R78 form a salt bridge and that this interaction is important for accurate initiation in a promoter-specific manner. Other defects in this region, including replacements at W63, R64, and F66, also affect start site selection (Bangur *et al.* 1997; Pardee *et al.* 1998). Among the new replacements described here, only C149Y affects initiation, causing a modest downstream shift at the *ADH1* promoter (Figure 3). All other replacements described in Table 2 had no discernable effect on the accuracy of initiation. The limited number of amino acid replacements that affect start site selection implies that specific defects, rather than general structural abnormalities, account for altered initiation.

The TFIIB E62K and R78C replacements markedly diminish the affinity of TFIIB for RNAPII as defined by surface plasmon resonance (Bushnell *et al.* 1996), suggesting that altered start sites might be a consequence of diminished TFIIB-RNAPII affinity. However, an R64E replacement affects start site selection, but does not affect TFIIB-RNAPII affinity (Bangur *et al.* 1997; Pardee *et al.* 1998). Instead, R64E enhances DNA-TBP-TFIIB ternary complex formation, although this was reported not to be a general effect of replacements that alter start site selection (Bangur *et al.* 1997; Pardee *et al.* 1998). Therefore, it appears that neither diminished TFIIB-RNAPII affinity nor enhanced ternary complex stability directly affects start site selection. The observed start site effects might be a consequence of altered interaction between the active site of RNAPII and the DNA template at the initiator region. Accordingly, specific TFIIB alterations would affect the active site of RNAPII such that it no longer efficiently recognizes certain start site sequences. In such cases RNAPII would scan downstream of normal, seeking preferable start sites. This scenario is consistent with evidence that RNAPII in *S. cerevisiae* is capable of scanning promoter DNA (Giardina and Lis 1993). Furthermore, downstream start sites are not new initiation sites, but correspond to enhanced initiation at normally weak initiation sites (Pinto *et al.* 1992, 1994; Berroteran *et al.* 1994). Thus, TFIIB defects do not alter the specificity of start site selection, but enhance the use of otherwise acceptable

sites whose use is precluded or diminished by upstream sites in a normal strain. A feature of this model is that altered start site selection occurs only at promoters where the normal start sites are relatively weak initiator elements and predicts that the normal pattern of initiation might be restored by *cis*-acting initiator mutations that create preferred start sites.

Interaction of TFIIB with Sub1 and Ssu72: Our results demonstrate that *ssu72-1* and *sub1*Δ interact with *sua7* in an allele-specific manner and that the specificity of these interactions is completely overlapping (summarized in Table 2). Furthermore, *sua7-1* does not enhance the growth defect of the temperature-sensitive *ssu72-2* mutant (encodes R129A; D. L. Pappas and M. Hampsey, unpublished results), suggesting that the interaction of *sua7-1* with *ssu72* is also allele specific. These results argue that TFIIB interacts directly with Ssu72 and Sub1. Indeed, Sub1 was shown previously to bind TFIIB (Knaus *et al.* 1996) and data presented here demonstrate direct binding of Ssu72 to TFIIB.

Although no specific function has been assigned to Ssu72, Sub1 is identical to Tsp1, identified as a factor that stimulates basal transcription *in vitro*, and interacts with both TFIIB and the activation domain of Gal4-VP16 (Henry *et al.* 1996). Furthermore, the N terminus of Sub1 is structurally similar to human PC4, a transcriptional coactivator that appears to bridge the interaction between gene-specific activators and the general transcriptional machinery (Ge and Roeder 1994; Kretzschmar *et al.* 1994). The role of PC4 in transcription is complex, repressing transcription under certain conditions, while stimulating transcription under others (Malik *et al.* 1998; Werten *et al.* 1998). PC4 binds single-stranded DNA, an activity that is dispensable for its activation function, but is required for repression in the absence of TFIIH (Werten *et al.* 1998). A dynamic role for PC4 has been proposed, involving conversion of an inactive promoter complex to an initiation-competent structure (Malik *et al.* 1998). Although less well characterized, the function of Sub1 is also complex, stimulating basal transcription *in vitro*, but facilitating transcriptional activation *in vivo*. Like PC4, Sub1 is phosphorylated and this modification controls its activity (Henry *et al.* 1996).

Despite the overlapping allele specificities of *ssu72-1* and *sub1*Δ with respect to *sua7* interactions, the Ssu72 and Sub1 proteins are not functionally redundant. There is no structural relationship between these two proteins and disruption of *SUB1* confers only minimal growth defects (Knaus *et al.* 1996), whereas *SSU72* is essential for cell viability (Sun and Hampsey 1996b). Guarente and co-workers proposed that Sub1 is a clearance factor that stimulates release of TFIIB from the promoter by disrupting the interaction between TFIIB and TBP (Knaus *et al.* 1996). On the other hand, Ssu72 was suggested to be an assembly factor that promotes formation of the preinitiation complex (Sun and Hampsey 1996b). Accordingly, Ssu72 and Sub1 appear to interact in a similar fashion with TFIIB, but do not necessarily influence the same event in transcription. In support of this idea, high-copy *SUB1* expression suppresses the Csm$^-$ growth defects of E62 and R78 mutants but does not restore the normal transcription start site pattern (W.-H. Wu and M. Hampsey, unpublished results). Furthermore, high-copy *SUB1* does not suppress the growth defects of *sua8/rpb1* mutations (B-S. Chen and M. Hampsey, unpublished results), even though *sua8* alleles cause the same defect in start site selection as the *sua7-1* allele (Berroteran *et al.* 1994).

Perspectives: The collection of TFIIB mutants described here is a valuable resource for extensive characterization of the role of TFIIB in transcription by RNAPII. These TFIIB derivatives can be used to correlate defects in the accuracy or rate of transcription *in vivo* with specific biochemical defects *in vitro*. For example, the TFIIB S53P mutant is defective for activation of the *PHO5* and *ADH2* genes *in vivo* and defective for interaction with the Pho4 and Adr1 activators *in vitro*, thereby implicating TFIIB as the physiological target of specific activators in yeast (Wu and Hampsey 1999). These mutants are also a valuable collection of primary mutants that can be exploited for genetic suppression analysis. Since all of the mutants described here display at least one conditional growth defect, revertants can be readily selected. The value of this type of analysis has already been demonstrated for the *sua7-1* mutant (Sun and Hampsey 1995, 1996b; Sun *et al.* 1996). Additional factors are likely to turn up as either suppressors or enhancers of other TFIIB defects and might define either novel components of the RNAPII transcriptional machinery or novel activities associated with known factors.

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