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Genetics

Identification of the gene (*SSU71/TFG1*) encoding the largest subunit of transcription factor TFIIF as a suppressor of a TFIIB mutation in *Saccharomyces cerevisiae*

(SSU71/transcription start-site selection)

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ABSTRACT **Mutations in the *Saccharomyces cerevisiae* *SSU71* gene were isolated as suppressors of a transcription factor TFIIB defect that confers both a cold-sensitive growth defect and a downstream shift in transcription start-site selection at the *cyc1* locus. The *ssu71-1* suppressor not only suppresses the conditional phenotype but also restores the normal pattern of transcription initiation at *cyc1*. In addition, the *ssu71-1* suppressor confers a heat-sensitive phenotype that is dependent upon the presence of the defective form of TFIIB. Molecular and genetic analysis of the cloned *SSU71* gene demonstrated that *SSU71* is a single-copy essential gene encoding a highly charged protein with a molecular mass of 82,194 daltons. Comparison of the deduced Ssu71 amino acid sequence with the protein data banks revealed significant similarity to RAP74, the larger subunit of the human general transcription factor TFIIF. Moreover, Ssu71 is identical to p105, a component of yeast TFIIF. Taken together, these data demonstrate a functional interaction between TFIIB and the large subunit of TFIIF and that this interaction can affect start-site selection *in vivo*.**

Accurate transcription initiation by RNA polymerase II (pol II) requires auxiliary factors that include the TATA-binding protein (TBP) and transcription factors TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH (1–3). These factors assemble in a defined order on a DNA template to generate a functional transcription preinitiation complex *in vitro* (4). A minimal transcription system composed of pol II, TBP, TFIIB, and TFIIF is necessary and sufficient for accurate basal transcription (5, 6), although TFIIF is dispensable for transcription from a supercoiled template (7). In the yeast *Saccharomyces cerevisiae*, several of these factors exist in a holoenzyme complex with pol II, consisting of the 12 pol II subunits and the Srb proteins, along with either TFIIB, TFIIF, and TFIIH (8) or TFIIF, Sug1, and Gal11 (9).

The functional role of the basal transcription factors and the mechanism of transcription initiation are the subjects of intense investigation. Mutations in the yeast genes encoding TFIIB (*SUA7*) and the largest subunit of pol II (*RPB1/SUA8*) can dramatically affect transcription start-site selection, thereby establishing a role for these two components in defining start sites *in vivo* (10, 11). Moreover, TFIIB and pol II appear to be the sole determinants of accurate start-site selection *in vitro*, since replacement of both TFIIB and pol II from *S. cerevisiae* by their counterparts from *Schizosaccharomyces pombe* is sufficient to switch start-site selection to the pattern characteristic of *S. pombe* (12).

Sequence analysis of TFIIB revealed three structural features: a zinc-finger motif near the N terminus, two imperfect repeats in the C-terminal two-thirds of the protein, and a potential amphipathic helix flanked by the repeats (10, 13). These elements are organized into structurally distinct domains defined as a compact, protease-resistant C-terminal core, and a protease-susceptible N-terminal region (14, 15). Mutational analysis demonstrated that the C-terminal core is necessary and sufficient for interaction with the TBP–promoter complex and that the N-terminal region interacts with pol II–TFIIF and is essential for basal transcription initiation (14–18). The N-terminal region also includes the most phylogenetically conserved region of TFIIB. This sequence lies immediately distal to the zinc finger and is required for accurate start-site selection *in vivo* (19).

TFIIF has been identified from human [RAP30/RAP74 (20)], rat [β/γ (21)], *Drosophila* [factor 5 (22)], and *S. cerevisiae* [factor g (23)]. Human TFIIF is composed of two subunits with apparent molecular masses of 30 kDa and 74 kDa (24), whereas factor g is composed of three subunits of 105 kDa, 54 kDa, and 30 kDa (23). A number of biochemical properties have been assigned to TFIIF, including high affinity for pol II (20, 22, 23, 25), stabilization of the preinitiation complex (26–29), accurate start site recognition (24), and the ability to stimulate the rate of elongation (22). The individual contributions of the RAP30 and RAP74 subunits to TFIIF functions are not well understood. Although RAP30 has been implicated in many of the functions attributed to TFIIF (5, 27, 30–32), the role of RAP74 has remained obscure. However, recent evidence indicates that both subunits are required for most functions attributed to TFIIF (29, 33).

We are using a genetic approach to identify components of the transcription preinitiation complex, emphasizing identification of factors that functionally interact with TFIIB. We recently reported that replacement of the phylogenetically invariant Glu-62 by lysine in TFIIB can have a dramatic effect on transcription start-site selection (19). In addition, the Glu-62 → Lys replacement confers a pronounced cold-sensitive (Csm^-) growth defect, a phenotype often associated with defects in assembly of multisubunit complexes (34). We have taken advantage of this Csm^- phenotype to isolate extragenic suppressors of the TFIIB defect. Here we report that mutations in the gene designated *SSU71** compensate for both the Csm^- phenotype and the altered initiation pattern conferred by the Glu-62 → Lys replacement. *SSU71* was identified as the gene encoding the p105 subunit of factor g. This information shows a functional interaction between TFIIB and the large subunit of TFIIF and shows that this interaction can affect start site selection *in vivo*.

MATERIALS AND METHODS

Yeast Strains, Genetic Methods, and Nomenclature. The yeast strains used in this study are listed in Table 1. Standard

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Abbreviations: pol II, RNA polymerase II; TBP, TATA-binding protein; Csm^-, cold-sensitive; Tsm^-, heat-sensitive.

*The sequence for *SSU71* reported in this paper has been deposited in the GenBank data base (accession no. U14527).

Table 1. Yeast strains, relevant genotypes, and phenotypes

Strain*	Relevant genotype	Phenotype†		
		Cyt *c*, %	Csm	Tsm
T16	*cyc1-5000*	<2	+	+
YDW546	*cyc1-5000 sua7-1*	30	−	+
YMH71-9C	*cyc1-5000 sua7-1*	30	−	+
YZS14	*cyc1-5000 sua7-1 ssu71-1*	<2	+	−
YZS45	*cyc1-5000 sua7-1 ssu71-2*	<2	+	−
YZS14-2B	*cyc1-5000 sua7-1 ssu71-1*	<2	+	−
YZS14-3B	*cyc1-5000 sua7-1 ssu71-1*	<2	+	−
YZS14 × YDW546	*cyc1-5000/cyc1-5000 sua7-1/sua7-1 SSU71/ssu71-1*	30	−	+

*Strains T16, YDW546, and YMH71-9C were described previously (10, 11); all others are described in this study.

†Phenotypes are defined as follows: Cyt *c* corresponds to the iso-1-cytochrome *c* content as determined by low-temperature, whole-cell spectroscopy (35) and is reported as a percentage of the iso-1-cytochrome *c* content in a *CYC1*$^+$ strain; Csm and Tsm refer to growth on rich (yeast extract/peptone/dextrose) medium at 16°C and 37°C, respectively.

procedures were used for crossing strains, selecting diploids, inducing sporulation, and dissecting tetrads (36). Standard yeast nomenclature is used throughout to designate both genotypes and phenotypes. For example, *ssu71-1* denotes a recessive allele of the wild-type *SSU71* gene. The symbols Csm$^-$ (cold-sensitive) and Tsm$^-$ (heat-sensitive) refer to distinctly impaired growth (or no growth) on rich medium (yeast extract/peptone/dextrose) at 16°C and 37°C, respectively. Cyt *c* in Table 1 corresponds to the iso-1-cytochrome *c* content as determined by low-temperature, whole-cell spectroscopy (35) and is reported as a percentage of the iso-1-cytochrome *c* content in a *CYC1*$^+$ strain.

Isolation of the *SSU71* Gene. The *SSU71* gene was isolated from a YCp50 genomic library (37) by complementation of the Tsm$^-$ phenotype conferred by the *ssu71-1* allele using strain YZS14 as the host. The relationship between the complementing DNA and the genetically defined *ssu71* locus was established by integration of the cloned DNA into the genome of strain YDW546 (*sua7 SSU71*$^+$ *leu2*) by homologous recombination (38). This tags the chromosomal locus of the cloned DNA with the *LEU2* marker. The resulting strain was crossed with strain YZS14-2B (*sua7 ssu71 leu2*), sporulated, and dissected. The *LEU2* marker segregated opposite of *ssu71* (Tsm$^-$) among all progeny. The *SSU71* gene was delimited by restriction analysis to a 3.2-kb *Kpn* I–*Sph* I DNA fragment (Fig. 1). Southern blot analysis confirmed that the 3.2-kb *Kpn* I–*Sph* I DNA fragment was isolated intact from the yeast genome and that *SSU71* is a single-copy gene (data not shown).

DNA Sequence Analysis. The sequences of both strands of *SSU71* were determined by the dideoxy-terminator method using the M13 universal primer or *SSU71*-specific primers and single-stranded DNA derived from exodeoxyribonuclease III deletions of *SSU71*.

Sequence Comparisons and Homologies. DNA and protein sequence comparisons were performed by using the BLAST algorithm (40), accessed via the National Center for Biotechnology Information E-mail service.

Determination of Transcript Start Sites. Primer extension was performed as described previously, using total RNA and the *cyc1*-specific primer oIP-13 (10). Primer extension products were resolved in an 8% polyacrylamide DNA sequencing gel and visualized by autoradiography.

RESULTS

Isolation of the *SSU71* Gene. The *sua7-1* allele of strain YMH71-9C encodes a TFIIB Glu-62 → Lys replacement that confers both altered start-site selection and a severe Csm$^-$

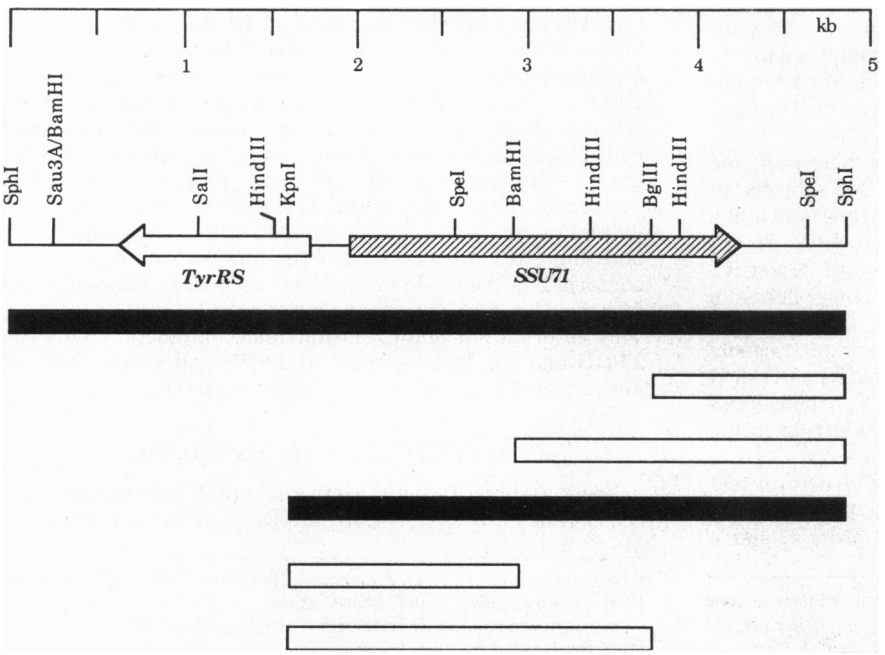

FIG. 1. Restriction map of the *SSU71* region. A partial restriction map of the *SSU71* locus is shown. DNA fragments that complement both the Tsm$^-$ and Csm$^+$ phenotypes associated with the *ssu71* suppressor in a *sua7-1* background are indicated by black bars, whereas open bars denote failure to complement. The complementing 3.2-kb *Kpn* I–*Sph* I fragment in the vector YCplac33 is designated pM435. The *TyrRS* gene, which encodes a tyrosine tRNA synthetase (39), lies immediately adjacent to *SSU71*.

phenotype (19). Spontaneous Csm$^+$ revertants of strain YMH71-9C were selected on rich medium at 16°C. When scored for potential pleiotropic phenotypes, 3 of 120 Csm$^+$ revertants were found to be Tsm$^-$, failing to grow at 37°C (Fig. 2). All three strains reacquired the Csm$^-$ and Tsm$^+$ phenotypes when crossed with a *sua7-1* mutant, indicating that both phenotypes are the result of recessive mutations (Fig. 2). A diploid strain derived from one of these revertants (YZS14; Table 1) was sporulated and dissected. The Csm$^+$ and Tsm$^-$ phenotypes cosegregated, thereby defining Tsm$^-$ as a marker for the suppressor of *sua7-1*.

The Tsm$^-$ phenotype was exploited to clone the suppressor gene. A yeast genomic library was introduced into strain YZS14, and transformants were selected at 37°C. Two classes of transformants were identified. Consistent with the anticipated phenotypes for the wild-type suppressor gene, one class of Tsm$^+$ transformants also reacquired the Csm$^-$ phenotype associated with *sua7-1*. When cured of plasmid DNA, these strains reacquired the Tsm$^-$ and Csm$^+$ phenotypes, indicating that the conditional phenotypes of the transformants are a consequence of plasmid DNA, rather than strain reversion. Plasmid DNA was recovered from these transformants, and subsequent genetic analysis established that the wild-type suppressor gene had indeed been cloned (see *Materials and Methods*). Plasmid pM435 restored the Tsm$^+$ and Csm$^-$ phenotypes to both YZS14 and YZS45, indicating that these two strains compensate for the *sua7-1* defect as a consequence of mutations in the same gene. This gene was designated *SSU71* (*s*uppressor of *sua7*, gene *1*), and the suppressor alleles were designated *ssu71-1* (YZS14) and *ssu71-2* (YZS45) (Table 1).

The second class of Tsm$^+$ transformants remained Csm$^+$. Analysis of plasmid DNA recovered from these transformants revealed a restriction pattern characteristic of the *SUA7* gene. This suggested that the Tsm$^-$ phenotype conferred by *ssu71* is dependent upon the *sua7-1* mutation. This was confirmed genetically. Whereas Tsm$^-$:Tsm$^+$ segregated 2:2 among the meiotic progeny from a cross of YZS14 to a *sua7-1* mutant (*sua7/sua7 SSU71/ssu71*), Tsm$^-$:Tsm$^+$ segregated at an overall ratio of 1:3 from a cross of YZS14 to a wild-type strain (*SUA7/sua7 SSU71/ssu71*). The dependency of the Tsm$^-$ phenotype associated with *ssu71* on the *sua7-1* allele implies a functional relationship between TFIIB and the product of *SSU71*.

The *SSU71* Sequence. The DNA sequence of *SSU71* identified an open reading frame encoding 735 amino acids, corresponding to a protein with a molecular mass of 82,194 daltons (Fig. 3). Consistent with the size of the coding region, RNA (Northern) blot analysis detected a single *SSU71* transcript of 2.3 kb (data not shown). The Ssu71 amino acid sequence was compared to the protein sequence data banks. The most significant similarities were to the RAP74 subunit of human, *Drosophila*, and *Xenopus* TFIIF. The similarity of Ssu71 and RAP74 is even more evident when considering

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MSRRNPPGSRNGGGPTNASPFIKRDRMRRN   30
FLRMRMGQNGSNSSSPGVPNGDNSRGSLVK   60
KDDPEYAEEREKMLLQIGVEADAGRSNVKV   90
KDEDPNEYNEFPLRAIPKEDLENMRTHLLK  120
FQSKKKINPVTDFHLPVRLHRKDTRNLQFQ  150
LTRAEIVQRQKEISEYKKKAEQERSTPNSG  180
GMNKSGTVSLNNTVKDGSQTPTVDSVTKDN  210
TANGVNSSIPTVTGSSVPPASPTTVSAVES  240
NGLSNGSTSAANGLDGNASTANLANGRPLV  270
TKLEDAGPAEDPTKVGMVKYDGKEVTNEPE  300
FEEGTMDPLADVAPDGGGRAKRGNLRRKTR  330
QLKVLDENAKKLRFEEFYPWVMEDFDGYNT  360
WVGSYEAGNSDSYVLLSVEDDGSFTMIPAD  390
KVYKFTARNKYATLTIDEAEKRMDKKSGEV  420
PRWLMKHLDNIGTTTTRYDRTRRKLKAVAD  450
QQAMDEDDRDDNSEVELDYDEEFADDEEAP  480
IIDGNEQENKESEQRIKKEMLQANAMGLRD  510
EEAPSENEEDELFGEKKIDEDGERIKKALQ  540
KTELAALYSSDENEINPYLSESDIENKENE  570
SPVKKEEDSDTLSKSKRSSPKKQQKKATNA  600
HVHKEPTLRVKSIKNCVIILKGDKKILKSF  630
PEGEWNPQTTKAVDSSNNASNTVPSPIKQE  660
EGLNSTVAEREETPAPTITEKDIIEAIGDG  690
KVNIKEFGKFIRRKYPGAENKKLMFAIVKK  720
LCRKVGNDHMELKKE*                735
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FIG. 3. The Ssu71 deduced amino acid sequence (single letter code). The asterisk signifies the *C* terminus.

other structural characteristics. Most strikingly, both Ssu71 and RAP74 are highly charged, consisting of 34% and 38% D, E, R, and K residues, respectively. Neither protein contains structural features commonly associated with many transcription factors, including leucine-zipper or zinc-finger motifs [the two proteins include only one (RAP74) or two (Ssu71) cysteine residues]. Both proteins contain multiple potential phosphorylation sites for cAMP-dependent protein kinases and protein kinase C, and these sites are concentrated in the C-terminal half of each protein (41). This information suggests a functional relationship between Ssu71 and RAP74.

***SSU71* Encodes the p105 Subunit of Yeast TFIIF.** Factor g is reported to be the yeast counterpart of human TFIIF and is composed of three polypeptides with apparent molecular masses of 30, 54, and 105 kDa (23). The genes encoding these three subunits were recently isolated and sequenced. The deduced N termini (14 residues) of the *TFG1*-encoded p105 subunit and the Ssu71 protein were compared; the two sequences were found to be identical (42). Subsequent comparison of the entire sequences confirmed that Ssu71 and p105 are indeed identical (42). Thus, *SSU71* is identical to *TFG1* and encodes the largest subunit of yeast TFIIF. The discrepancy between the deduced (82 kDa) and apparent (105 kDa) molecular masses of the Ssu71/p105 protein is presumably due to anomalous migration in SDS/PAGE. Highly charged proteins are known to exhibit anomalously slow migration in SDS/PAGE (43), and RAP74 is such a protein, with a calculated molecular mass of 58 kDa and an apparent molecular mass of 74 kDa (41, 44). Although the slower mobility of RAP74 might be a consequence of posttranslational modification, recombinant RAP74 synthesized in *Escherichia coli* also migrates at 74 kDa (44).

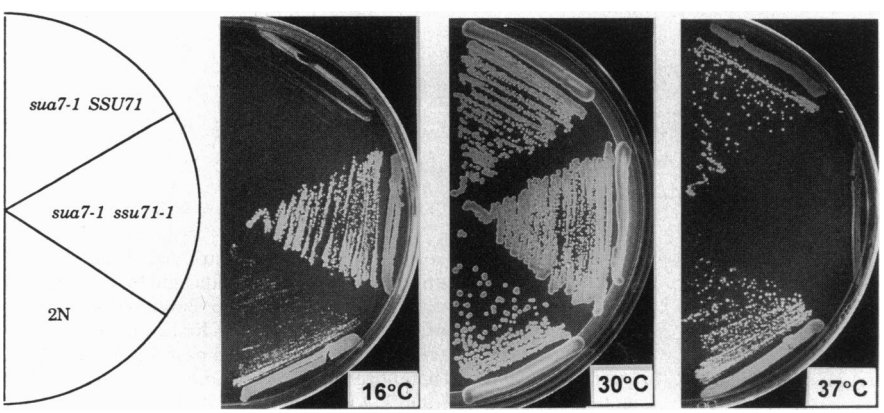

FIG. 2. Phenotypes associated with *sua7* and *ssu71* mutants. Growth of strains YMH71-9C (*sua7-1 SSU71*), YZS14 (*sua7-1 ssu71-1*), and a diploid strain (2N) derived from a cross of YZS14 with YDW546 (*sua7-1 SSU71*) is shown on yeast extract/peptone/dextrose (YPD) at reduced (16°C), normal (30°C), and elevated (37°C) temperatures. YZS14 was selected as a spontaneous revertant of YMH71-9C on YPD medium at 16°C. Whereas YMH71-9C is phenotypically Csm$^-$ and Tsm$^+$, YZS14 is Csm$^+$ and Tsm$^-$. Both Csm$^+$ and Tsm$^-$ are the result of recessive mutation(s), since the Csm$^-$ and Tsm$^+$ phenotypes are restored when YZS14 is backcrossed with strain YDW546.

***SSU71* is Essential for Cell Viability.** The *SSU71* open reading frame was disrupted by insertion of the *LEU2* gene into the *Bam*HI site (Fig. 1), and the resulting construct was used to disrupt one allele of *SSU71* in a homozygous *leu2*$^-$ diploid strain. Upon sporulation and dissection, viable-to-inviable spores segregated at a ratio of 2:2 (14 tetrads), and all viable spores were phenotypically Leu$^-$ (data not shown). Four-spore viability was restored when the diploid strain was transformed with plasmid pM435 (*SSU71*) prior to dissection. These experiments define *SSU71* as an essential gene.

The *ssu71* Suppressor Restores Normal Start-Site Selection in an *sua7* Background. The *sua7-1* mutation confers a marked effect on transcription start-site selection. Since the Csm$^-$ phenotype and start site effects are a consequence of the same mutation (10, 19), we asked whether the *ssu71-1* suppressor also compensates for the start-site shift. Restoration of the normal initiation pattern in the *ssu71* strains was anticipated based on their iso-1-cytochrome *c* phenotypes. These strains carry the *cyc1-5000* allele, which is the result of an aberrant ATG translation start codon [upstream ATG (uATG), position −20] in the transcribed leader region of *cyc1-5000* (10). This mutation diminishes iso-1-cytochrome *c* synthesis to <2% of normal. The *sua7-1* mutation partially suppresses this defect by shifting transcription initiation downstream of the uATG, enhancing the iso-1-cytochrome *c* level to ≈30% of normal (10). However, the iso-1-cytochrome *c* level was restored to <2% of normal in the *ssu71* mutants (Table 1), suggesting that initiation had shifted back upstream of the uATG.

An effect of *ssu71* on start-site selection was confirmed by mapping transcription start sites at the *cyc1* gene by primer extension analysis. Consistent with previous results, *sua7-1* enhanced initiation at downstream sites, including discrete sites between positions −28 and −14 (Fig. 4, lanes 1 vs. 2). The normal initiation pattern was restored in the *ssu71-1* mutant (Fig. 4, lane 3), and this effect cosegregated with *ssu71-1* (Fig. 4, lane 4). Thus, *ssu71-1* does not simply compensate for Csm$^-$ but also influences start-site selection, presumably through interaction, either direct or indirect, with TFIIB.

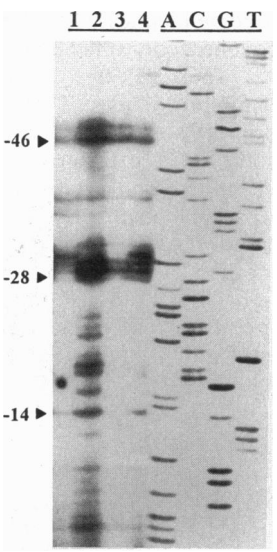

FIG. 4. Primer extension analysis of *cyc1* transcription start sites. Lanes: 1, T16 (*SUA7 SSU71*); 2, YMH71-9C (*sua7-1 SSU71*); 3, YZS14 (*sua7-1 ssu71-1*); 4, YZS14-3B (*sua7-1 ssu71-1*). Strain YZS14-3B is a segregant of a backcross of YZS14 with YDW546 (Table 1). Transcription start sites are numbered relative to the ATG translation start codon. The sequence ladder (A,C,G,T) corresponds to *SUA7* DNA and was used as a molecular size marker. Whereas initiation is enhanced at downstream sites in the *sua7* mutant relative to the wild-type strain (compare lanes 1 and 2), the normal initiation pattern is restored in the isogenic *sua7 ssu71* strain (lane 3); this effect cosegregates with the *ssu71* marker (lane 4).

DISCUSSION

The data presented in this paper establish a functional interaction between TFIIB and the largest subunit of TFIIF in yeast. First, the *SSU71* gene, which encodes the largest subunit of TFIIF, was uncovered as a suppressor of a Glu-62 → Lys replacement (*sua7-1*) in TFIIB. Second, *sua7 ssu71* double mutants exhibit a synthetic phenotype: double *sua7 ssu71* mutants are heat-sensitive, whereas neither *sua7 SSU71*$^+$ nor *SUA7*$^+$ *ssu71* single mutants exhibit a phenotype at 37°C. Finally, the distinct downstream shift in transcription start-site selection at the *cyc1* locus associated with the *sua7-1* allele is fully suppressed by the *ssu71-1* allele, resulting in the normal pattern of initiation at *cyc1*.

Glu-62 is an invariant residue within the most phylogenetically conserved region of TFIIB. This region and the adjacent zinc-finger motif are included within the proteolytically susceptible domain of TFIIB that is required for recruitment of pol II–TFIIF to the promoter–TBP–TFIIB complex and for transcription initiation (14–18). TFIIF is thought to mediate the interaction between pol II and the promoter–TBP–TFIIB complex and this function has been attributed specifically to the RAP30 subunit (27). Consistent with this premise, TFIIB binds to RAP30, and this interaction has been mapped to the N-terminal domain of TFIIB (18). However, suppression of the Glu-62 → Lys replacement in yeast TFIIB by a mutation in the gene encoding a homolog of RAP74 suggests that interaction between TFIIB and TFIIF involves both subunits of TFIIF. However, there is currently no evidence for a direct interaction between TFIIB and either RAP74 or Ssu71/p105; efforts to characterize the interaction between TFIIB and Ssu71/p105 have been hampered by the toxicity associated with expression of *SSU71* derivatives in *E. coli* (unpublished observation).

Activities attributed to TFIIF are reminiscent of those assigned to the σ^{70} family of bacterial transcription factors, including tight binding to pol II, suppression of nonspecific binding of pol II to the free DNA, and stabilization of the preinitiation complex (45). Furthermore, limited sequence similarities to σ^{70} have been noted for both RAP30 and RAP74 (46–48). Comparison of the Ssu71/p105 and RAP74 sequences to *E. coli* σ^{70} revealed two regions of sequence similarity corresponding to the σ^{70} RpoD box, which straddles homology regions 2.3 and 2.4, and to homology region 4.2 (Fig. 5). This observation is particularly noteworthy because regions 2.4 and 4.2 are involved in recognition of the −10 and −35 regions, respectively, of the bacterial promoter (49). The question of whether there is functional significance to these sequence similarities can now be readily investigated by mutational analysis of the cloned *SSU71* gene.

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                σ RpoD box
421   EYRRGYKFST---YATWWIRQAITR   σ70
        ::  |||::   |||  |::|  |
388   PADKVYKFTARNKYATLTIDEAEKR   Ssu71/p105
      |::: |:||:  :  |||::|||::
139   PVHNWYNFTPLARHRTLTAEEAEEE   RAP74

              σ region 4.2
572   TLEEVGKQFDVTRERIRQIEAKALRK  σ70
      ||:  : :  |   : ::  :|: :
607   TLRVKSIKNCVIILKGDKKILKSFPE  Ssu71/p105
      |||: : |     |:  |:: : . |
400   TLRAAASK-----LEQGKRVSEM-PA  RAP74
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FIG. 5. Alignment of *E. coli* σ70, yeast Ssu71/p105, and human RAP74. Sequence identity and similarity are indicated by vertical lines and colons, respectively. Similarity rules are P═A═S, G═A═S, T═A═S, D═E═N═Q═K═R═H, A═V═I═L, and F═Y═W. The *E. coli* RpoD box is underlined and encompasses portions of σ^{70} regions 2.3 and 2.4. The entire region 4.2 of σ^{70} is underlined. The similarity between σ^{70} and RAP74 was noted previously (48).

The mechanism of transcription start-site selection is not well characterized, although TFIIB and pol II are the sole determinants of accurate start-site selection in a yeast *in vitro* system (12). It is noteworthy that mutations in both TFIIB (*sua7*) and the largest subunit of pol II (*sua8*) exert nearly identical downstream shifts in start-site selection (10, 11). Furthermore, all *sua7* and *sua8* mutants are extremely Csm^-, a phenotype often associated with defects in the assembly or stability of multisubunit complexes (34). Perhaps the effects of *sua7* and *sua8* mutations on start-site selection are a consequence of impaired ability to form a stable preinitiation complex at the initiation site. In yeast, initiation generally occurs at multiple sites located typically 30–120 bp from TATA. Yet promoter melting and pol II entry occur at a fixed distance of ≈20 bp from TATA, suggesting that the pol II complex binds the promoter and then "scans" for suitable start sites (50). In the *sua7* and *sua8* mutants, the assembly or stability of the complex might be altered such that scanning proceeds further downstream of normal. The data presented here suggest that the Ssu71/p105 subunit of TFIIF is a component of the scanning preinitiation complex and that the *ssu71-1* mutation might stabilize the complex to restore the normal initiation pattern. This would be consistent with the established role of human TFIIF in stabilization of the transcription preinitiation complex—perhaps by promoting DNA–protein contacts between the TATA element and the transcription start site—and in formation of the first phosphodiester bond (24, 26, 28, 33).

Z.-W. S. dedicates this paper to the memory of his father, Mu-Chern Sun. We are grateful to N. L. Henry and R. D. Kornberg (Stanford Univ.) for communicating results prior to publication and to Judith Jaehning (University of Colorado Health Sciences Center, Denver), Zach Burton (Michigan State University), and Mark Goebl (Indiana University–Purdue University, Indianapolis) for valuable discussions. This work was supported by grants from the National Institutes of Health (GM-39484) and the American Cancer Society (NP-842).

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