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Synthetic Enhancement of a TFIIB Defect by a Mutation in *SSU72*, an Essential Yeast Gene Encoding a Novel Protein That Affects Transcription Start Site Selection In Vivo

ZU-WEN SUN† AND MICHAEL HAMPSEY*

Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, Louisiana 71130

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An *ssu72* mutant of *Saccharomyces cerevisiae* was identified as an enhancer of a TFIIB defect (*sua7-1*) that confers both a cold-sensitive growth defect and a downstream shift in transcription start site selection. The *ssu72-1* allele did not affect cold sensitivity but, in combination with *sua7-1*, created a heat-sensitive phenotype. Moreover, start site selection at the *ADH1* gene was dramatically shifted further downstream of the normal sites. Both of these effects could be rescued by either *SUA7* or *SSU72*, thereby defining a functional relationship between the two genes. *SSU72* is a single-copy, essential gene encoding a novel protein of 206 amino acids. The *ssu72-1* allele is the result of a 30-bp duplication creating a sequence encoding a Cys-X_2-Cys-X_6-Cys-X_2-Cys zinc binding motif near the N terminus of Ssu72p. Mutational analysis demonstrated that the N terminus of Ssu72p is essential for function and that cysteine residues in both the normal and mutant proteins are critical. We discuss the possibility that the potential zinc binding motif of Ssu72p facilitates assembly of the transcription preinitiation complex and that this effect is important for accurate start site selection in vivo.

Transcription initiation by RNA polymerase II (RNAP II) involves the concerted action of a large number of proteins. One class is gene specific and binds to promoters containing specific target sequences (reviewed in references 33 and 52). These factors stimulate the rate of transcription in response to specific physiological or developmental stimuli. In contrast, the general transcription factors are required for accurate, basal-level transcription in the absence of gene-specific stimulation (reviewed in references 6 and 77). With the exception of the TATA-binding protein (TBP), the general factors do not bind to specific DNA sequences. A third class of factors, known as coactivators, mediators, or adaptors, functions in communication between the gene-specific and general factors (reviewed in reference 25). This class includes the TBP-associated factors that, along with TBP, make up TFIID (26), as well as other adaptors, including the Ada2p-Ada3p-Gcn5p complex in *Saccharomyces cerevisiae* (31). Transcriptional activity is also affected by the chromatin structure of the DNA template (reviewed in reference 48). Both biochemical and genetic experiments suggest that one function of the gene-specific factors is to overcome the repressive effects of chromatin on transcription initiation. The identities of these factors and the mechanisms by which they interact to confer accurate, regulated initiation by RNAP II are the subject of intense investigation and are critical for understanding regulated cell growth and development.

The general transcription factors were initially defined in biochemical studies and include TBP (initially as a component of the TFIID complex), TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, and TFIIJ (reviewed in references 11 and 76). These factors assemble on a DNA template in a defined order to generate a functional transcription preinitiation complex in vitro (7, 71). Assembly of the complex is nucleated by association of TBP (or TFIID) with the TATA element, followed by binding of TFIIB. TBP physically interacts with TFIIB, and the crystal structure of a TFIIB-TBP-TATA element ternary complex was recently described (45). RNAP II, in association with TFIIF, then binds the complex. TFIIE and TFIIH associate with the complex after binding by RNAP II and are required for promoter clearance in a manner that is dependent upon ATP hydrolysis (27). Although there is considerable evidence to support this scheme for assembly of the preinitiation complex in vitro, RNAP II appears to be recruited to promoters in vivo as a holoenzyme complex that includes general factors. There is direct support for this premise in both yeast and mammalian systems, where holoenzyme complexes of somewhat different compositions have been reported (35, 37, 47).

Genetic studies using the yeast *S. cerevisiae* have provided valuable new information regarding the general transcription factors and their functions. For example, mutations in the *SPT* genes were identified as suppressors of Ty or solo δ insertions that inhibit transcription of adjacent *HIS4* or *LYS2* genes (72). One of these genes, *SPT15*, encodes TBP (15, 29). Mutations in *SPT15* shift initiation from the δ initiation site to the normal initiation site at *HIS4*, thereby establishing a role for TBP in normal transcription initiation in vivo (15). Another class of genes, designated *SUA*, were identified as suppressors of an aberrant open reading frame located in the transcribed leader region of the *CYC1* gene (50). Two of these genes, *SUA7* and *SUA8* (*RPB1*) encode, respectively, TFIIB and the largest subunit of RNAP II (4, 50). Mutations in either gene shifted transcription start site selection downstream of the normal sites. These experiments demonstrated that TFIIB and the largest RNAP II subunit are required for accurate transcription start site selection in vivo and suggested that specific interactions between TFIIB and the largest RNAP II subunit affect this process (4).

Genetic analysis of transcription initiation also has the potential to identify transcription factors that might go undetect-

* Corresponding author. Present address: Department of Biochemistry, Robert Wood Johnson Medical School, UMDNJ, 675 Hoes Ln., Piscataway, NJ 08854-5635.

† Present address: Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854-5635.

TABLE 1. Yeast strains used in this study

Strain	Genotype
T16	*MAT*α *cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2*
YDW546	*MAT*α *cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua7-1*
YMH71-9C	*MAT***a** *cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1*
YZS19	*MAT***a** *cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssu72-1 ssu73-1*
YZS19/YCplac33	*MAT***a** *cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssu72-1 ssu73-1* [*URA3*]
YZS19/pDW11	*MAT***a** *cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssu72-1 ssu73-1* [*URA3 SUA7*]
YZS19/pM385	*MAT***a** *cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssu72-1 ssu73-1* [*URA3 SSU72*]
YZS19/pM586	*MAT***a** *cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssu72-1 ssu73-1* [*URA3 SSU72*]
YZS19/pM681	*MAT***a** *cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssu72-1 ssu73-1* [*URA3 SSU73*]
YZS19-1B	*MAT***a** *ura3-52 leu2-3,112 sua7-1 ssu72-1*
YMH171	*MAT*α *ura3-52 leu2-3,112 his3 trp1*Δ
YZS89A	*MAT*α *ura3-52 leu2-3,112 his3 trp1*Δ *ssu72*::*LEU2* [pM586: *URA3 SSU72*]
LRB535/536	*MAT***a**/*MAT*α *his3*Δ*200/his3*Δ*200 leu2-3,112/leu2-3,112 ura3-52/ura3-52*

ed by biochemical means. For example, the *SPT3* (13), *SPT7* (21), and *SPT8* (14) genes encode novel factors that play important roles in normal transcription initiation yet were not detected biochemically. The products of these three genes are functionally related to TBP, and Spt3p has been shown to physically interact with TBP (13). Furthermore, human $TAF_{II}18$ is structurally similar to the N-terminal region of Spt3p (42). Another important example of transcription factors that were initially identified by genetic means are the Srb proteins. Mutations in the *SRB* genes were selected on the basis of their ability to suppress growth phenotypes associated with truncation of the heptapeptide repeat domain (CTD) located at the C terminus of the largest subunit of RNAP II (46). These factors are components of the RNAP II holoenzyme and include novel proteins associated with the preinitiation complex (36, 68) as well as a kinase-cyclin pair that is involved in phosphorylation of the RNAP II CTD (39). The *SPT*, *SUA*, and *SRB* systems underscore the importance of genetic approaches for identifying critical factors involved in transcription initiation by RNAP II.

We are continuing to use genetic methods to identify factors that are important for transcription initiation by RNAP II. One of our approaches is to isolate and characterize suppressors of defects in TFIIB (*sua7*) or in the largest subunit of RNAP II (*sua8*) that affect start site selection. The *sua7-1* allele encodes a TFIIB Glu-62→Lys (E62K) replacement that is responsible for both the aberrant initiation pattern and a cold-sensitive (*cs*$^-$) growth defect (51). By selecting for revertants of the *sua7-1 cs*$^-$ phenotype, we have uncovered three genes designated *ssu71*, *ssu72* and *ssu73* (for suppressors of *sua*7, genes 1, 2, and 3). *SSU71* is identical to *TFG1*, the gene encoding the largest subunit of TFIIF (64), and *SSU73* is identical to *RPB9*, the gene encoding the 14.2-kDa subunit of RNAP II (66). The *ssu71* and *ssu73* suppressors are functionally similar in that both not only compensate for the *cs*$^-$ phenotype but also restore the normal initiation pattern in the *sua7-1* background (64, 66). Here, we describe the *SSU72* gene. In contrast to the *ssu71* and *ssu73* suppressors, *ssu72-1* is an enhancer of the *sua7-1* defect, conferring a synthetic heat-sensitive phenotype (*ts*$^-$) and causing start site selection at the *ADH1* and *CYC1* genes to shift further downstream of the normal sites. Genetic and molecular analyses demonstrated that *SSU72* is an essential gene encoding a novel protein that might affect the assembly of the transcription preinitiation complex.

MATERIALS AND METHODS

Yeast strains and media. The yeast strains used in this study are listed in Table 1. Strains T16 and YDW546 were described previously (50). YMH71-9C is a *sua7-1* segregant derived from YDW546 (44). YZS19 is a spontaneous *cs*$^+$ revertant of YMH71-9C. YZS19/YCplac33 (*sua7 ssu72 ssu73*), YZS19/pDW11 (*SUA7 ssu72 ssu73*), YZS19/pM385 and YZS19/pM586 (*sua7 SSU72 ssu73*), and YZS19/pM681 (*sua7 ssu72 SSU73*) are derivatives of YZS19 carrying the indicated plasmids. YZS19-1B is an *sua7 ssu72 SSU73* segregant derived from a backcross of YZS19 with YDW546. Strain YZS89A (*SUA7 ssu72*::*LEU2* [*URA3-SSU72*]) was used as the host in the plasmid shuffle system and was derived from YMH171 as described below. LRB535/536 is a diploid strain used as the host for disruption of *SSU72* (65).

All media were prepared according to standard procedures (59). YPD refers to rich medium consisting of 1% yeast extract, 2% peptone, and 2% glucose. 5-FOA medium contains 5-fluoro-orotic acid and was used as a positive selection against the *URA3*$^+$ gene (5).

Genetic methods and nomenclature. Standard procedures were used for crossing yeast strains, selecting diploids, inducing sporulation, and dissecting tetrads (60). Yeast transformations were done by the lithium acetate procedure (23). Plasmid DNA was recovered from *S. cerevisiae* as described previously (30) and introduced into *Escherichia coli* by electroporation (12). All other transformations of *E. coli* were done by the calcium chloride procedure (40). *E. coli* XL1-Blue (*recA1 endA1 gyrA96 thi-1 supE44 hsdR17* (r_K^- m_K^-) *relA1 lac* [F′ *proAB lacI*^{q} *lacZ*Δ*M15* Tn*10* (Tet^r)]) was used as the host strain for all plasmids.

Standard yeast genetic nomenclature is used throughout, with upper- and lowercase italicized symbols denoting dominant and recessive alleles, respectively (e.g., *SSU72* and *ssu72-1*). The symbols Ura$^-$, Leu$^-$, and Trp$^-$ denote failure to grow on media lacking Ura, Leu, and Trp (−Ura, −Leu, and −Trp media, respectively). Cold- and heat-sensitive growth phenotypes are expressed as *cs*$^-$ and *ts*$^-$, respectively, and denote distinctly impaired growth (or no growth) on rich medium (YPD) at 16 and 37°C.

Plasmids. Restriction sites pertaining to *SSU72* constructs are depicted in Fig. 2. The low-copy-number vectors YCp50 (*CEN URA3*) (55), pRS314 (*CEN TRP1*) and pRS316 (*CEN URA3*) (62), and YCplac33 (24); the integrating vector pRS305 (*LEU2*) (62); and the high-copy-number vector YEp24 (2 μm *URA3*) (55) have been described previously. The plasmids were constructed as follows. (i) pM264 is the 2.3-kb *Sal*I-*Sph*I fragment encompassing *SUA7* in the high-copy-number vector YEp24. (ii) pM384 is the original *SSU72* clone carrying a ~12.5-kb DNA insert in the vector YCp50. (iii) pM385 is the 1.05-kb *Eco*RI-*Hin*dIII *SSU72* DNA fragment in pRS316. (iv) pM471 is the 3.8-kb *Hin*dIII-*Hin*dIII *SSU72* DNA fragment in the vector pRS316. (v) pM471A is identical to pM471, except that the *Hin*dIII fragment was inserted into pRS316 in the opposite orientation. (vi) pM523 was derived from pM539 by replacement of the *Hin*dIII-*Bcl*I DNA fragment encompassing the entire *SSU72* open reading frame with a *LEU2 Hin*dIII-*Bam*HI DNA fragment. (vii) pM524 is *ssu72* DNA lacking the sequence encoding the N-terminal 23 amino acids and was amplified by PCR with primers that generate flanking *Eco*RI and *Spe*I sites. Amplified DNA was digested with *Eco*RI and *Spe*I and ligated into YCpIF17 (18). This construct expresses an HA-Ssu72 protein, lacking the N-terminal 23 residues of Ssu72p, behind the *GAL1* promoter. (viii) pM539 is the 2.0-kb *Apa*I-*Bam*HI *SSU72* DNA fragment in pRS316. (ix) pM540 is identical to pM539, except that it carries the *ssu72-1* allele. pM540 was recovered from strain YZS19 by gap repair using pM539 that had been digested with *Eco*RI and *Hin*dIII. (x) pM550 is identical to pM524, except that it encodes full-length Ssu72p as an HA fusion protein behind the *GAL1* promoter. (xi) pM552 is the 2.7-kb *Xba*I-*Hin*dIII DNA fragment encompassing *SSU72* and *ORF498* in the *LEU2* integrating vector pRS305. (xii) pM586 is the 1.05-kb *Eco*RI-*Hin*dIII *SSU72* DNA fragment in YCplac33. (xiii) pM681 is the *RPB9* (*SSU73*) gene in the YCplac33 vector. The 3.2-kb *Pst*I-*Bam*HI fragment encompassing *RPB9* was transferred from plasmid Ro406 (20) to YCplac33. (xiv) pM699 includes the entire *SSU72* open reading frame and was amplified by PCR with oligonucleotides that create flanking *Xma*I-*Nco*I sites and inserted in the *CUP1*-glutathione *S*-transferase (GST) vector pCBGST-1 (41), allowing for expression of a GST-Ssu72 fusion protein under *CUP1* control. (xv)

pM712 is the 1.05-kb *Eco*RI-*Kpn*I DNA fragment encompassing the *SSU72* wild-type gene in the *TRP1* vector pRS314. (xvi) pM740 is identical to pM699, except that the N-terminal 4 residues of Ssu72p were eliminated from the GST-Ssu72 fusion protein and the restriction sites flanking *SSU72* are *Not*I and *Nco*I. (xvii) pM742 is identical to pM740, except that the N-terminal 10 amino acids of Ssu72p were eliminated from the GST-Ssu72 fusion protein. (xviii) pDW11 is the *SUA7* wild-type gene in the vector pRS316 (50).

Isolation of the *SSU72* gene. The *SSU72* gene was isolated from a YCp50 genomic library (56) by complementation of the ts^- phenotype conferred by the *ssu72-1* allele of strain YZS19. The relationship between the complementing DNA and the genetically defined *ssu72* locus was established by integration of the cloned DNA into the genome of strain YDW546 (*sua7 SSU72*$^+$ *leu2*) by homologous recombination (55). Plasmid pM552, which carries the 2.7-kb *Xba*I-*Hin*dIII DNA fragment encompassing *SSU72*, was digested at the unique *Bam*HI site within the open reading frame adjacent to *SSU72*. Linearized pM552 was then introduced into YDW546, and transformants were selected on −Leu medium. This tags the chromosomal locus of the cloned DNA with the *LEU2* marker, which was confirmed by Southern blot analysis (data not shown). This strain was crossed with YZS19-1B (*sua7 ssu72 leu2*), and a resulting diploid was sporulated and dissected. Prior to mating, the *SUA7* gene was introduced into YZS19-1B on a *URA3* high-copy-number plasmid (pM264); the presence of the *SUA7* wild-type gene improved the sporulation and germination efficiencies of homozygous *sua7* diploids. Segregants were cured of the plasmid-borne *SUA7* gene on 5-FOA medium and subsequently scored for Leu and *ts* phenotypes. Complete cosegregation of the Leu$^+$/ts^+ and Leu$^-$/ts^- phenotypes confirmed that the cloned gene is *SSU72*.

DNA sequence analysis. Plasmids pM471 and pM471A were linearized at the *Sac*I and *Not*I sites within the multiple cloning region, and nested sets of deletions were generated with exonuclease III and S1 nuclease (Erase-a-Base Kit; Promega). Single-stranded DNA was generated from selected clones with the VCSM13 helper phage (Stratagene Cloning Systems) in the presence of kanamycin, as described previously (58). Both strands of the entire 3,785-bp DNA fragment were sequenced by the dideoxy-terminator method with the M13 universal primer; sequencing gaps were filled in with *SSU72*-specific primers. The entire open reading frame of the *ssu72-1* allele was sequenced with single-stranded DNA derived from plasmid pM540.

Construction of the *ssu72*::*LEU2* null allele. Plasmid pM523 was digested with *Apa*I and *Bam*HI, and the resulting *ssu72*::*LEU2* DNA fragment was introduced into diploid strain LRB535/536, selecting for Leu$^+$ transformants. Integration at one of the two *SSU72* loci, resulting in replacement of the *SSU72* gene with *LEU2* (*ssu72*::*LEU2*), was confirmed by Southern blot analysis (data not shown).

Determination of transcript start sites. Primer extension was performed as described previously, using total RNA and the *ADH1*-specific primer oIP-87 (50). Primer extension products were resolved in 6% polyacrylamide DNA sequencing gels and visualized with a Molecular Dynamics PhosphorImager. The molecular size markers were *SUA7* DNA that had been sequenced with the primer oIP-133 (50).

Isolation of the *ssu72-1* allele. The *ssu72-1* allele was cloned from strain YZS19 by gap repair (57). Plasmid pM539 was digested with *Eco*RI and *Hin*dIII, thereby deleting the entire *SSU72* open reading frame (see Fig. 2). The gapped vector was purified following size fractionation in an agarose gel and introduced into YZS19. Ura$^+$ transformants were selected and screened for retention of the ts^- phenotype associated with *ssu72-1*. Plasmid DNA was recovered and digested with *Eco*RI and *Hin*dIII, confirming recovery of *ssu72* DNA. This plasmid was designated pM540.

In vitro construction of *ssu72* alleles. Site-directed mutations in *SSU72* were generated with synthetic oligonucleotides by PCR according to a technique described previously (43). Template DNA was either plasmid pM539 (*SSU72*) or pM540 (*ssu72-1*). Following amplification, DNA was digested with *Eco*RI (*SSU72* site) and *Kpn*I (vector site) and inserted into the *TRP1* vector pRS314 (62). Plasmids expressing N-terminal deletions of Ssu72p (4-, 10-, and 23-nucleotide deletions) were constructed as described above.

Functional analysis of *ssu72* alleles. A plasmid shuffle system (61) was set up to assess the function of in vitro-generated *ssu72* alleles. The chromosomal *SSU72* locus of strain YMH171 (*SUA7*$^+$ *SSU72*$^+$) was disrupted with the *Apa*I-*Bam*HI DNA fragment encompassing *ssu72*::*LEU2* from plasmid pM523. Strain viability was maintained by introducing plasmid pM586 (*URA3 SSU72*) into YMH171 prior to disruption. The resulting strain is designated YZS89A (Table 1). Phenotypes associated with in vitro-constructed *ssu72* alleles were assessed by introducing *ssu72 TRP1* plasmids into YZS89A and subsequently curing Trp$^+$ transformants of pM586 (*URA3 SSU72*) on 5-FOA medium. Failure to recover 5-FOA-resistant (FOA^r) transformants indicated that the *ssu72* allele is nonfunctional. FOA^r strains were subsequently scored for growth on rich medium (YPD). Plasmids pM712 (*SSU72*), pM699 (*CUP1*-GST-*SSU72*), and pM550 (*GAL1*-HA-*SSU72*) served as the positive controls in these experiments.

Sequence comparisons and homologies. DNA and protein sequence comparisons were performed with the BLAST algorithm (2), accessed via the National Center for Biotechnology Information electronic mail service.

Nucleotide sequence accession number. The 3.8-kb DNA sequence encompassing *SSU72* has been entered in the GenBank data library under accession number U20390.

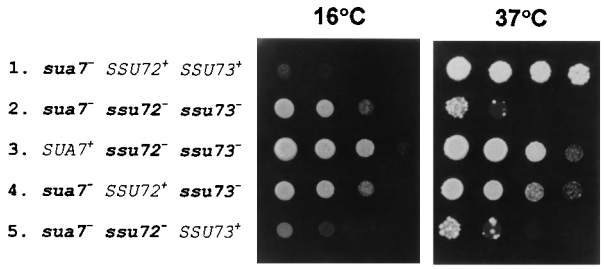

FIG. 1. Conditional growth defects associated with *sua7*, *ssu72*, and *ssu73* mutations. Strain YZS19 (*sua7 ssu72 ssu73*) is a spontaneous cs^+ derivative of YMH71-9C (*sua7 SSU72 SSU73*). The strains depicted in rows 2 to 5 were derived from YZS19 by introducing low-copy-number plasmids carrying either *SUA7*, *SSU72*, or *SSU73* or vector alone. Consequently, all strains are isogenic, differing only by the indicated alleles. Strain names are as follows: YMH71-9C (*sua7 SSU72 SSU73*), YZS19/YCplac33 (*sua7 ssu72 ssu73*), YZS19/pDW11 (*SUA7 ssu72 ssu73*), YZS19/pM586 (*sua7 SSU72 ssu73*), and YZS19/pM681 (*sua7 ssu72 SSU73*). These results demonstrate that *ssu72-1* is an enhancer of the *sua7-1* allele, conferring a severe ts^- phenotype (cf. row 5 and row 1), whereas *ssu73-1* is a suppressor of the cs^- phenotype associated with *sua7-1* (cf. row 4 and row 1). The phenotypes associated with the *ssu72-1* and *ssu73-1* mutations are absolutely dependent upon the *sua7-1* allele, since the *SUA7* plasmid rescues all phenotypes (row 3). Columns represent cell growth of serial dilutions (left to right). The revertant colonies seen in rows 2 and 5 at 37°C are indicative of the high spontaneous reversion frequency of the *ssu72-1* allele.

RESULTS

Isolation of an *ssu72* mutant. The *sua7-1* allele encodes an E62K replacement in TFIIB that confers both altered start site selection and a severe cs^- phenotype (51) (Fig. 1, row 1). Cold sensitivity is often associated with defects in assembly of multisubunit complexes (reviewed in reference 73). This suggests that the *sua7-1* mutant might be defective in assembly of the transcription preinitiation complex. Consistent with this possibility, the form of TFIIB encoded by *sua7-1* is functional and exhibits normal stability in vivo (51). Consequently, suppressors or enhancers of the E62K form of TFIIB might include factors that either facilitate or impair complex assembly.

One hundred twenty spontaneous cs^+ revertants of an *sua7-1* mutant (YMH71-9C) were isolated. In an effort to identify potential pleiotropic phenotypes, all cs^+ revertants were scored for ts^-. Three revertants were found to be severely ts^-, failing to grow on rich medium (YPD) at 37°C. Two of these strains, YZS14 and YZS45, are the result of recessive mutations in *SSU71* (*TFG1*), the gene encoding the largest subunit of TFIIF (64). Genetic analysis of the third strain, YZS19, revealed that the cs^+ and ts^- phenotypes are the result of recessive mutations in two unlinked genes, designated *ssu72-1* and *ssu73-1*. The *ssu73-1* mutation suppressed the cs^- phenotype conferred by *sua7-1* (66), whereas the *ssu72-1* mutation in combination with *sua7-1* is responsible for the ts^- phenotype (Fig. 1).

Isolation of the *SSU72* gene. The ts^- phenotype of strain YZS19 was used to clone the *SSU72* wild-type gene. A YCp50 yeast genomic library (56) was introduced into YZS19, and transformants were selected at 37°C. Twenty-three ts^+ transformants were isolated from a total of ~60,000 Ura$^+$ transformants. Plasmid DNA was recovered from 12 transformants and reintroduced into YZS19, confirming that the ts^+ phenotype of each strain is conferred by plasmid DNA rather than strain reversion. Restriction analysis identified three distinct plasmids with a common 3.8-kb *Hin*dIII restriction fragment. One of these plasmids, designated pM384, was chosen for further analysis.

Plasmid pM384 carries an insert of approximately 12.5 kb in the vector YCp50. The gene responsible for complementation

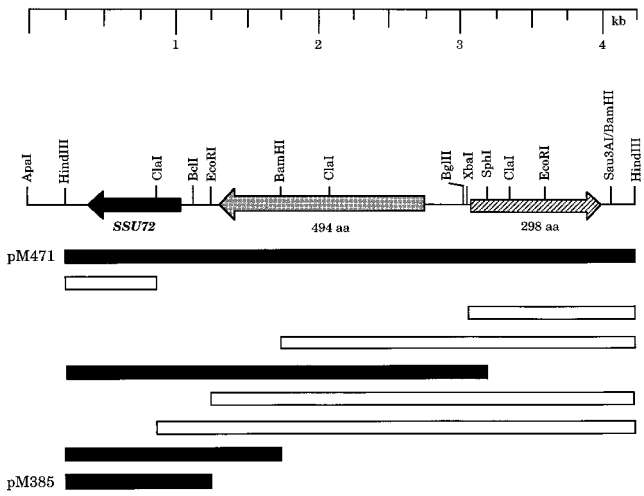

FIG. 2. Restriction map of the *SSU72* region. A partial restriction map encompassing the *SSU72* gene is shown. DNA fragments that complement the *ts*$^-$ phenotype associated with the *ssu72-1* suppressor in the *sua7-1* background are indicated by black bars, whereas open bars denote failure to complement. The 3.8-kb *Hin*dIII-*Hin*dIII and 1.05-kb *Eco*RI-*Hin*dIII DNA fragments in the vector pRS316 (62) are designated pM471 and pM385, respectively. The adjacent open reading frames encode potential proteins of 494 and 298 amino acids (aa) with unknown functions.

of *ts*$^-$ was initially delimited to the common 3.8-kb *Hin*dIII DNA fragment (plasmid pM471) (Fig. 2). Southern blot analysis confirmed that this fragment was derived intact from the yeast genome and that *SSU72* is a single-copy gene (data not shown). The relationship between the complementing DNA and the genetically defined *ssu72* gene was established by integration of the cloned DNA as part of a *LEU2* vector into the genome of YDW546. This strain (*sua7 leu2 SSU72*::*LEU2*) was crossed with strain YZS19-1B (*sua7 leu2 ssu72*), and a resulting diploid was sporulated and dissected. A total of 18 segregants were obtained and scored. All Leu$^+$ progeny were phenotypically *ts*$^+$, and all Leu$^-$ progeny were *ts*$^-$. Segregation of the *LEU2*-tagged locus opposite to the *ssu72-1* marker (*ts*$^-$) established that the cloned gene is indeed *SSU72*.

Genetic relationship between *SUA7* and *SSU72*. Independent segregation of the *cs*$^+$ (*ssu73*) and *ts*$^-$ (*ssu72*) phenotypes from YZS19 raises the question of whether *SSU72* is functionally related to *SUA7*. As described above, *SSU72* was cloned by complementation of the *ts*$^-$ phenotype. In addition, plasmid DNA from 9 of 12 *ts*$^+$ transformants of YZS19 exhibited a *Hin*dIII restriction pattern that is characteristic of the *SUA7* gene. Plasmid pDW11, which carries *SUA7* on a low-copy-number plasmid (50), was subsequently found to transform YZS19 to *ts*$^+$. The ability of either the *SUA7* or the *SSU72* wild-type gene to restore *ts*$^+$ demonstrates that the *ts*$^-$ phenotype associated with *ssu72-1* is dependent upon *sua7-1*. Therefore, the *ts*$^-$ phenotype of YZS19 is not simply a consequence of the *ssu72-1* mutation but is a "synthetic" phenotype requiring both *sua7-1* and *ssu72-1*. This effect is reminiscent of the *ssu71-1* suppressor, which is similarly dependent upon the *sua7-1* mutation for the associated *ts*$^-$ phenotype (64). As for *SSU71*, this is an extremely important result because it demonstrates a genetic relationship between TFIIB and the product of the *SSU72* gene. Thus, strain YZS19 contains both a suppressor (*ssu73-1*) and an enhancer (*ssu72-1*) of the same mutation (*sua7-1*). These two mutations were found in a single strain as a consequence of selecting for one phenotype (*cs*$^+$) and screening for the other (*ts*$^-$).

We recently identified the *ssu73-1* suppressor as an allele of *RPB9* (66). Thus, all three genes represented in YZS19 (*SUA7*, *SSU72*, and *SSU73*/*RPB9*) have been cloned. This enabled us to confirm the genetically defined relationships among these genes by introducing low-copy-number vectors carrying *SUA7*, *SSU72*, or *SSU73* into strain YZS19 and scoring the resulting transformants for growth at 16 and 37°C. The results are shown in Fig. 1. Strain YZS19/pM586 (*sua7 SSU72 ssu73*) is both *cs*$^+$ and *ts*$^+$ (row 4), confirming that *ssu73-1* alone suppresses the *sua7-1 cs*$^-$ phenotype but does not confer *ts*$^-$. In contrast, YZS19/pM681 (*sua7 ssu72 SSU73*) exhibits the same *cs*$^-$ phenotype as the parent strain YMH71-9C (*sua7 SSU72 SSU73*) (cf. rows 1 and 5), confirming that *ssu72-1* is not a suppressor of *sua7-1*. However, the *ts*$^-$ phenotype of YZS19 is retained in the presence of the *SSU73* gene (cf. rows 2 and 5) yet is eliminated by either *SSU72* or *SUA7* (rows 3 and 4), confirming dependence of *ts*$^-$ on both *sua7-1* and *ssu72-1*. These results firmly establish that *ssu72-1* is an enhancer and *ssu73-1* is a suppressor of the same TFIIB defect.

The *SSU72* sequence. The entire 3.8-kb *Hin*dIII fragment encompassing *SSU72* was sequenced. Three open reading frames of 618, 1,482, and 894 nucleotides were identified (Fig. 2). The *SSU72* gene was subsequently delimited to a 1.05-kb *Eco*RI-*Hin*dIII DNA fragment (pM385) that includes only the 618-nucleotide open reading frame (Fig. 3). Accordingly, *SSU72* encodes a potential protein of 206 amino acids with a calculated molecular mass of 23,469 Da. Ssu72p is acidic (pI = 4.9) and enriched in charged residues (27% D, E, K, and R). The N terminus includes a half-site C-X_2-C potential metal binding motif (see below). Two imperfect direct repeats were identified (residues 69 to 78 and 82 to 90), suggesting an internal element of symmetry within Ssu72p. These structural features are summarized in Fig. 4. No structural features commonly associated with transcription factors were found, although there is a clustering of acidic residues in the C-terminal half of the protein.

The Ssu72p sequence was compared with the protein sequence databases by using the BLAST algorithm (2). Although no homologous proteins were found, similarity to a conserved region of ATP-dependent RNA helicases was identified. The similarity between Ssu72p and the consensus residues within this region was especially good (Fig. 4). Nonetheless, the Ssu72p sequence includes neither the DEAD box of ATP-binding proteins nor the highly conserved HRIGRXXR motif required for RNA binding and ATP hydrolysis (49). Consequently, Ssu72p is unlikely to be an ATP-dependent RNA helicase.

***SSU72* is an essential gene.** The *SSU72* locus was disrupted by replacing the *Hin*dIII-*Bcl*I fragment encompassing the entire *SSU72* open reading frame with a *LEU2 Hin*dIII-*Bam*HI DNA fragment (Fig. 2). The resulting construct was used to disrupt one copy of *SSU72* in the homozygous *leu2* diploid strain LRB535/536. Upon sporulation and dissection, no more than two viable spores were recovered from each tetrad (Fig. 5) and all viable spores were phenotypically Leu$^-$. Inspection of inviable segregants under the microscope revealed that most had germinated, undergoing two to three cell divisions before cessation of growth. Four-spore viability was restored when the diploid strain was transformed with plasmid pM385 (*SSU72 URA3*) prior to dissection. When the resulting segregants were replica printed onto 5-FOA medium, selecting against the plasmid-borne copy of *SSU72*, two of four segregants, all of which were Leu$^-$, grew (data not shown). Therefore, *SSU72* is essential for cell growth.

The *ssu72-1* mutation affects transcription start site selection. Since the *sua7-1* mutation shifts transcription start site se-

```
          EcoRI
-233      GAATTCACTAGTTCATAAGCATATATACTTGTTTTAATATATTCCCTAAAACT
-180 GAGGTATCCAGTTCTTTTGTTCTTATTCGTTTGATCATCCTTTACGTATTTTTCGTCTGC
-120 GGCAGTGGAAAGAACTTAAAAAACGAAAGATAATAAAGAAAAAGGCCAGTCGGCACGAAT
 -60 TAAAGACCACCTGTAAGAAGATTAAGACAACGGTGGTATAAACTAGTGTCGAAGTCCAAA
   1 ATGCCTAGTCATCGCAATTCAAACTTGAAGTTTTGCACAGTTTGTGCATCAAACAACAAT
   1 M P S H R N S N L K F C T V C A S N N N

  61 CGTTCAATGGAATCGCATAAAGTCCTGCAAGAAGCAGGCTATAATGTTAGCTCTTACGGA
  21 R S M E S H K V L Q E A G Y N V S S Y G

 121 ACAGGTTCAGCTGTGAGACTGCCTGGTCTATCGATAGATAAGCCTAATGTGTACTCATTT
  41 T G S A V R L P G L S I D K P N V Y S F

 181 GGTACACCCTATAATGATATATATAATGATCTTTTATCACAATCAGCAGACCGTTACAAG
  61 G T P Y N D I Y N D L L S Q S A D R Y K

 241 TCGAACGGTTTATTGCAAATGCTGGATCGTAATAGAAGACTCAAAAAAGCACCTGAAAAA
  81 S N G L L Q M L D R N R R L K K A P E K

 301 TGGCAAGAAAGTACAAAAGTCTTCGACTTCGTTTTCACTTGTGAAGAGAGATGTTTTGAT
 101 W Q E S T K V F D F V F T C E E R C F D

 361 GCCGTTTGTGAAGATTTGATGAATAGAGGTGGGAAATTAAACAAAATAGTGCATGTAATT
 121 A V C E D L M N R G G K L N K I V H V I

 421 AATGTTGACATTAAAGATGATGATGAAAATGCTAAAATTGGTAGCAAAGCTATATTGGAA
 141 N V D I K D D D E N A K I G S K A I L E

 481 TTAGCTGATATGCTCAATGATAAAATAGAACAATGTGAAAAAGATGACATTCCCTTTGAA
 161 L A D M L N D K I E Q C E K D D I P F E

 541 GATTGTATAATGGACATTTTAACTGAGTGGCAAAGCTCACATTCTCAACTACCGTCATTA
 181 D C I M D I L T E W Q S S H S Q L P S L

 601 TACGCTCCTTCATATTACTAAACAAAACGGTCAATTACTGTAGAAAAGCATAAGCATTAA
 201 Y A P S Y Y *

 661 GCGGCCCTCATGAATCCTACATTATCCGTGAAATTTCAATACTTTCACAAGTCTTCGATA
 721 GCTTCCTAGTTATATTCATTATATAATATTACGTTTCTTGTTGCGGCTCTCTTAAGCACA
                                        HindIII
 781 TTATTTGCTTGTTGTCCACATAGCTATTTTTTTATCAAGAAGCTT
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FIG. 3. The *SSU72* sequence. The complete nucleotide sequence of the 1,058-bp *Eco*RI-*Hin*dIII DNA fragment encompassing *SSU72* is shown, along with the deduced amino acid sequence of Ssu72p. The *Eco*RI site (−233) is located 6 nucleotides downstream of the termination codon of ORF494 (Fig. 2). The 6-bp direct repeats that flank the duplicated sequence in the *ssu72-1* mutant (nucleotides 25 to 54), the corresponding amino acid sequence that is duplicated (residues 9 to 18), and the region of sequence similarity to ATP-dependent RNA helicases (residues 122 to 150) are underlined. The imperfect amino acid repeats (residues 69 to 78 and 82 to 90) are indicated by arrows, and identical residues are indicated in boldface type.

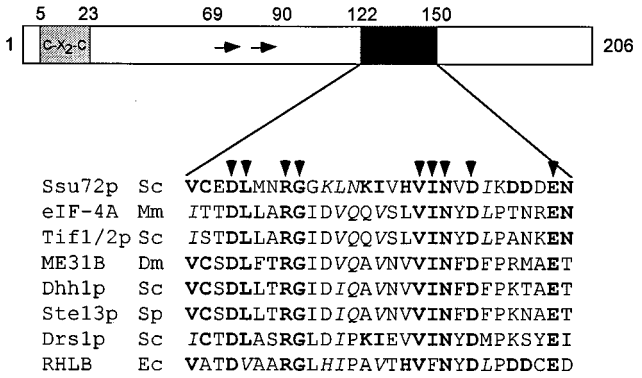

FIG. 4. Structural features of Ssu72p. The essential N terminus (residues 5 to 23) is denoted by the shaded box and includes the sequence C-X_2-C, which is duplicated in the *ssu72-1* mutant. The imperfect repeats (Fig. 3) are denoted by arrows. The black box (residues 122 to 150) denotes sequence similarity to known or putative ATP-dependent RNA helicases. This region is aligned with eIF-4A from mice; Tif1 and Tif2, the putative counterparts of eIF-4A from yeasts; ME31B, a maternally expressed *Drosophila* gene; Dhh1p, Ste13p, and Drs1p, putative helicases from *S. cerevisiae* and *S. pombe*; and RHLB, a putative helicase from *E. coli*. Sc, Mm, Dm, Sp, and Ec denote *S. cerevisiae*, *Mus musculus*, *Drosophila melanogaster*, *S. pombe*, and *E. coli*, respectively. Residues that are identical to Ssu72p are highlighted in boldface type, and conserved residues are highlighted in italics. Arrowheads denote residues that are identical among the eukaryotic proteins (excludes *E. coli* RHLB).

lection downstream of the normal sites (50), we asked whether *ssu72-1* might also affect this process. Transcription start sites were mapped at the *ADH1* locus by primer extension (Fig. 6). Transcription of the *ADH1* gene normally initiates at two sites located at positions −27 and −37 relative to the ATG start codon (Fig. 6, lane 2). Consistent with previous results (50), the *sua7-1* mutation diminished initiation at position −37 and enhanced initiation at multiple sites located downstream of −27 (Fig. 6, lane 1). Strikingly, in strain YZS19, initiation was nearly eliminated at −37 and reduced at −27 yet was dramatically enhanced at multiple sites located further downstream (Fig. 6, lane 3). This effect is dependent upon both the *sua7-1* and *ssu72-1* mutations, since the normal initiation pattern was restored by introducing a low-copy-number plasmid carrying either the *SSU72* (Fig. 6, lane 4) or *SUA7* (lane 5) wild-type gene into YZS19. A similar enhanced downstream shift was observed at *CYC1* (data not shown), establishing that the *ssu72* effect on start site selection is not specific for the *ADH1* promoter. These results demonstrate that Ssu72p is involved in start site selection in a TFIIB-dependent manner and provide further evidence for a functional relationship between these two proteins.

The plasmid-borne *SSU72* gene in strain YZS19/pM385 (*sua7-1 ssu72-1*/*SSU72 ssu73-1*) restored the normal pattern of initiation at *ADH1* (Fig. 6, lane 4) rather than the pattern associated with the *sua7-1* defect (Fig. 6, lane 1). Since strain YZS19 is also mutated at the *ssu73* locus, the normal initiation pattern in the *SSU72* transformant suggests that *ssu73-1* compensates for the downstream shift caused by *sua7-1*, a result that was observed previously for the *ssu71-1* suppressor (64). Furthermore, both *ssu71-1* and *ssu73-1*, but not *ssu72-1*, suppress the *sua7-1 cs*$^-$ phenotype. Therefore, the *ssu71* and *ssu73* suppressors appear to be functionally related to each other and distinct from the *ssu72-1* enhancer.

Characterization of the *ssu72-1* mutation. The *ssu72-1* allele was cloned by gap repair (57), and the entire open reading frame was sequenced. A 30-bp duplication of nucleotides 25 to 54 was identified as the only mutation (Fig. 7). The *ssu72-1* duplication maintains the open reading frame and creates a 10-amino-acid direct repeat (or partially overlapping 12-amino-acid repeat) (Fig. 8). Interestingly, this repeat includes the amino acid sequence C-T-V-C such that a Cys_4 zinc binding motif (C-X_2-C-X_6-C-X_2-C) is created near the N terminus of

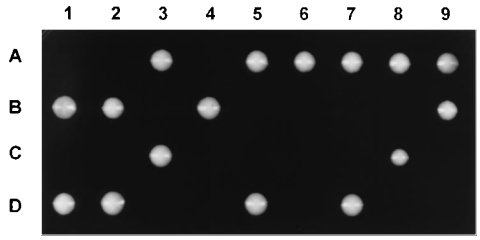

FIG. 5. *SSU72* is an essential gene. One copy of the *SSU72* gene was disrupted with the *LEU2* marker in the diploid strain LRB535/536 (*ssu72*::*LEU2*/*SSU72 leu2*/*leu2*). Upon sporulation and dissection, no more than two viable spores were recovered from each tetrad (9 of 25 tetrads are depicted) and all viable spores are phenotypically Leu$^-$. Four-spore viability was restored when dissections were done in the presence of the plasmid-borne *SSU72* gene (data not shown). When the resulting segregants were replica printed onto 5-FOA medium, selecting against plasmid-borne *SSU72*, two of four segregants, all of which were Leu$^-$, grew. These results establish that *SSU72* is an essential gene.

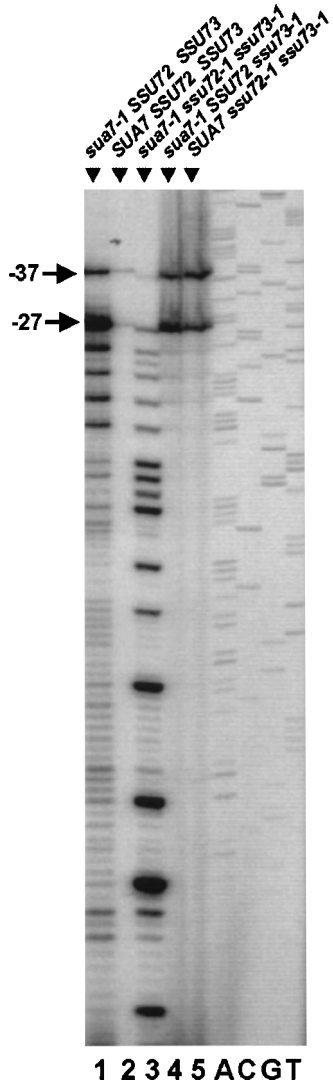

FIG. 6. Primer extension analysis of *ADH1* transcription start sites. Lane 1, YMH71-9C (*sua7-1*); lane 2, T16 (wild type); lane 3, YZS19 (*sua7-1 ssu72-1 ssu73-1*); lane 4, YZS19/pM385 (*sua7-1 SSU72 ssu73-1*); lane 5, YZS19/pDW11 (*SUA7 ssu72-1 ssu73-1*). Lanes A, C, G, and T were used as molecular size markers and correspond to a sequence ladder of *SUA7* DNA. Transcription initiates at comparable rates at positions −37 and −27 (A of the ATG start codon is designated +1) in the wild-type strain (lane 2). The *sua7-1* mutation causes a downstream shift in start site selection (lane 1), and this effect is exacerbated in the *sua7-1 ssu72-1 ssu73-1* mutant, in which the normal initiation sites are nearly eliminated in favor of multiple, discrete downstream sites (lane 3). The normal initiation pattern is restored in the presence of either the *SSU72* (lane 4) or *SUA7* (lane 5) gene, demonstrating that the downstream shift in YZS19 is dependent upon both the *sua7-1* and *ssu72-1* mutations. The normal initiation pattern is observed for the *sua7-1 SSU72 ssu73-1* strain, indicating that *ssu73-1* compensates for the downstream shift associated with *sua7-1* (cf. lanes 1 and 4). This effect is similar to that of the *ssu71-1* suppressor (64) and is in stark contrast to the effect of *ssu72-1*.

the altered form of Ssu72p. This is an intriguing result, because this motif has been implicated in protein-DNA or protein-protein interactions (53). Moreover, all three factors shown previously to affect start site selection in *S. cerevisiae* (TFIIB and the Rpb1p and Rpb9p subunits of RNAP II) contain C-X_2-C-X_{6-17}-C-X_2-C/H zinc binding motifs.

We investigated the importance of the Ssu72p N terminus by generating specific *ssu72* mutations in vitro and assessing their phenotypes by using a plasmid-shuffle system in vivo. The host strain carries the *SUA7* wild-type gene, thereby allowing for phenotypes to be attributed exclusively to *ssu72* mutations. The results are shown in Table 2. Deletion of the N-terminal 4 residues was without effect, but deletion of 10 or 23 N-terminal residues abolished Ssu72p function. Therefore, the N terminus of Ssu72p, which includes the duplicated sequence encoded by *ssu72-1*, is critical for function.

We next asked if the cysteine at position 15 is critical. Two mutations, one encoding a serine replacement (C15S) and the other encoding a histidine replacement (C15H), were created. The C15S mutant was inviable, whereas the C15H mutant grew nearly as well as the wild-type strain. The inviability of the C15S mutant establishes that amino acid 15 is critical, and the viability of the C15H mutant suggests that the C-X_2-C sequence might be involved in zinc chelation. We also generated comparable replacements of the cysteine at position 25 encoded by *ssu72-1* (Fig. 8). Despite retention of the normal C-15, the C25S mutant was inviable, whereas the C25H replacement was without effect. These data establish that cysteine residues located near the N terminus of both Ssu72p and the *ssu72-1*-encoded protein are essential for function and suggest that these residues are involved in zinc chelation.

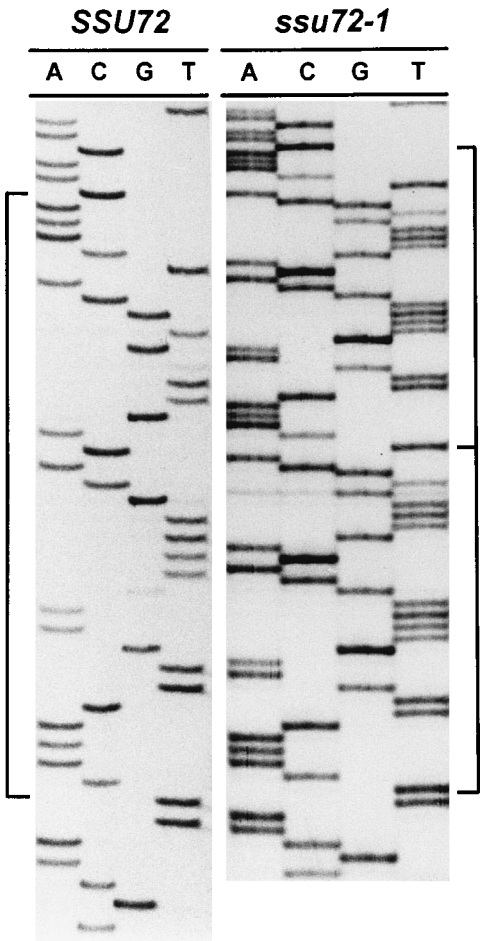

FIG. 7. DNA sequence of the *ssu72-1* allele. Partial DNA sequences of the *SSU72* and *ssu72-1* alleles are shown, in both cases from CGCAAT (base pairs 13 to 18) to AACAAT (base pairs 55 to 60) (Fig. 3). The brackets encompass the region of *SSU72* that was duplicated to create the *ssu72-1* allele and includes the TCAAAC sequence that flanks the reiterated sequence.

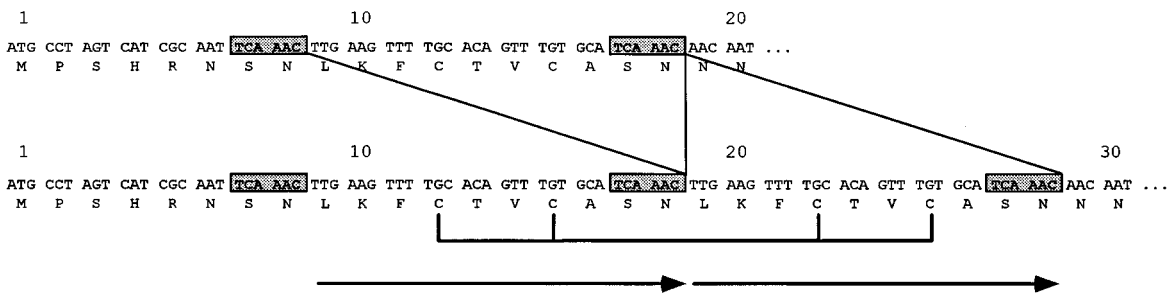

FIG. 8. The *ssu72-1* allele encodes a direct repeat near the N terminus of Ssu72p. The *ssu72-1* allele is the result of a 30-bp tandem duplication (Fig. 7). The 6-bp direct repeats that flank the duplication are boxed. The 10-amino-acid direct repeats resulting from the *ssu72-1* duplication are indicated by arrows. The cysteine residues within the repeated sequence form a zinc binding motif (C-X_2-C-X_6-C-X_2-C).

DISCUSSION

Functional relationship of Ssu72p to TFIIB. The *SSU71*, *SSU72*, and *SSU73* genes were uncovered in a genetic selection for suppressors of a conditional growth defect (*cs*$^-$) associated with a TFIIB mutation (*sua7-1*) that also alters transcription start site selection. The potential of this selection to identify genes whose products are functionally related to TFIIB was established by the identity of the *SSU71* gene. *SSU71* encodes a homolog of mammalian RAP74 and is identical to *TFG1*, the gene encoding the largest subunit of yeast TFIIF (64). The *ssu71-1* suppressor also compensated for the transcription initiation defect associated with *sua7-1*, restoring the normal pattern of start site selection at the *CYC1* gene. The *SSU73* gene is identical to *RPB9*, which encodes the 14.2-kDa subunit of RNAP II (66). Like *ssu71-1*, *ssu73-1* compensates for the *cs*$^-$ growth defect of *sua7-1* (Fig. 1) and restores the normal pattern of transcription start site selection at *ADH1* (Fig. 6, lane 4). These results imply a functional relationship between the largest subunit of TFIIF and the Rpb9p subunit of RNAP II.

The *ssu72-1* allele, in contrast to *ssu71* and *ssu73*, is not a suppressor of the *sua7-1 cs*$^-$ phenotype. Rather, *ssu72-1* arose spontaneously with *ssu73-1* in strain YZS19 and was identified on the basis of the *ts*$^-$ phenotype. Although this suggests that *SSU72* might be unrelated to *SUA7*, two results indicate otherwise. First, the *ssu72-1 ts*$^-$ phenotype requires the presence of the *sua7-1* allele; neither *SUA7 ssu72-1* nor *sua7-1 SSU72* single mutants are *ts*$^-$. This effect is defined as synthetic enhancement and indicates a genetic relationship between the two genes (28). Second, transcription start site selection is shifted significantly downstream of the normal sites in the YZS19 mutant, and this effect can be rescued by either *SUA7* or *SSU72* (Fig. 6, cf. lane 3 with lanes 4 and 5). These results demonstrate that Ssu72p can affect transcription initiation in a TFIIB-dependent manner in vivo and provide strong support for a functional relationship between the two proteins. Nonetheless, we have been unable to detect a direct interaction between these two proteins either by the two-hybrid system or by association of TFIIB with either GST-Ssu72p or HA-Ssu72p fusion proteins (63). Therefore, we do not know whether the functional relationship between TFIIB and Ssu72p involves a physical interaction.

TABLE 2. Viability associated with Ssu72p alterations

Ssu72p alteration	Viability[a]
N-term-Δ4[b]	Viable
N-term-Δ10[b]	Inviable
N-term-Δ23[b]	Inviable
C15S[c]	Inviable
C15H[c]	Viable
C25S[d]	Inviable
C25H[d]	Viable

[a] Strains expressing the indicated Ssu72p alterations were constructed by plasmid shuffling as described in Materials and Methods. Viability was assessed by the ability of Trp$^+$ transformants of YZS89A to grow on 5-FOA medium. FOA^r colonies were purified by subcloning and subsequently restreaked on rich medium (YPD) to compare growth rates with those of control strains expressing *SSU72*$^+$. Growth rates of viable mutants and wild-type control strains were comparable.

[b] The indicated alterations denote, respectively, deletion of the N-terminal 4, 10, and 23 residues of Ssu72p. These proteins were expressed from plasmids pM740 (Δ4) or pM742 (Δ10) as GST-Ssu72 fusion proteins or from plasmid pM524 (Δ23) as an HA-Ssu72 fusion protein. Cells expressing full-length GST-Ssu72 (pM699) or HA-Ssu72 (pM550) fusion proteins were viable, demonstrating that the fusion proteins are functional.

[c] Amino acid replacements are denoted by the single letter code. Accordingly, C15S and C15H designate, respectively, cysteine→serine and cysteine→histidine replacements at position 15.

[d] Amino acid replacements were generated by using the *ssu72-1* allele as the template. Therefore, the indicated replacements of C-25 occur within the 10-residue duplication encoded by *ssu72-1* (Fig. 8).

The primary structure of Ssu72p does not offer any clue to its function. The most significant sequence similarity is to several known and putative ATP-dependent RNA helicases. Although this similarity appears to be significant (Fig. 4), it is limited to a small region of the protein. The absence of other motifs that are characteristic of ATP-dependent RNA helicases implies that Ssu72p is not an RNA helicase. Rather, the sequence similarity suggests that Ssu72p and ATP-dependent RNA helicases share a common, albeit undefined, function.

The *ssu72-1* mutation. The *ssu72-1* allele is the result of a 30-bp duplication flanked by 6-bp direct repeats (Fig. 8). Consequently, *ssu72-1* encodes a 10-amino-acid repeat that creates a C-X_2-C-X_6-C-X_2-C zinc binding motif (Cys_4) near the N terminus of Ssu72p (Fig. 8). This result is particularly intriguing. The largest subunit of RNAP II contains a similar zinc binding motif (C-X_2-C-X_6-C-X_2-H) (1). This structure is located N terminal to the amino acid replacements encoded by the *sua8* alleles of *RPB1*, which exert effects on start site selection similar to those of *sua7-1* (4). TFIIB also includes a zinc binding motif (C-X_2-C-X_{17}-C-X_2-C) near its N terminus, and the *sua7-1*, *sua7-2*, and *sua7-3* alleles, all of which shift transcription start site selection downstream of the normal sites, encode single amino acid replacements in a highly conserved domain immediately adjacent to this motif (51). The Rpb9p subunit of RNAP II includes two zinc binding motifs, one near the N terminus and the other near the C terminus (74). Like *sua7* (TFIIB), *sua8* (Rpb1p), and *ssu72*, alleles of *RPB9* also affect start site selection. In these cases, start site selection was shifted upstream of the normal site and attributed either to an *rpb9* null allele or to replacement of a cysteine residue within

the N-terminal zinc binding motif (20, 32) or to a nonsense mutation immediately following the C-terminal zinc binding motif (66). All of these results point to a role for zinc binding domains in transcription start site selection, although the specific functions of the zinc binding regions remain undefined.

The $C\text{-}X_2\text{-}C\text{-}X_{(6–25)}\text{-}C\text{-}X_2\text{-}C$ motif has been found in other transcription factors, including the second largest subunit of RNAP II (67), TFIIE (16), elongation factor TFIIS (53), and the adenovirus E1A protein (22). A three-dimensional nuclear magnetic resonance structure revealed that the zinc binding domain of TFIIS does not form the classical zinc finger motif. Rather, TFIIS forms a three-stranded, antiparallel β-sheet, designated a zinc ribbon (53). It was suggested that this motif is inaccessible (cryptic) in the native protein but becomes exposed upon binding of TFIIS to an elongation complex. This is an interesting possibility and is reminiscent of the proposal that TFIIB exists in a latent form in which the N-terminal domain forms an intramolecular interaction with the C-terminal domain (54). Upon binding of a transcriptional activator, the zinc binding motif at the TFIIB N terminus is exposed, providing a surface for interaction with RNAP II-TFIIF. The zinc binding motif is critical for this function, since cysteine replacements eliminated the ability of TFIIB to recruit RNAP II-TFIIF into the preinitiation complex (8).

It should be noted that the *ssu72-1*-encoded protein, and not the normal Ssu72p, includes the $C\text{-}X_2\text{-}C\text{-}X_6\text{-}C\text{-}X_2\text{-}C$ zinc binding motif. Nonetheless, cysteine 15 at the essential N terminus of Ssu72p can be functionally replaced by histidine but not by serine, suggesting that the $C\text{-}X_2\text{-}C$ sequence is involved in zinc chelation. There is a precedent for this possibility. The Rpb10p subunit of RNAP II contains a similar half-site $C\text{-}X_2\text{-}C$ sequence (75) and is known to bind zinc (69). If the N terminus of Ssu72p does bind zinc, perhaps amino acids other than cysteine constitute the other two zinc ligands. Alternatively, Ssu72p might exist as a dimer that creates a zinc binding domain in *trans*, as proposed for the human immunodeficiency virus Tat protein (19).

There might appear to be a discrepancy between the structure of the *ssu72-1*-encoded protein and its function. The *ssu72-1* mutation creates, rather than eliminates, a structural motif (Cys_4) within the altered protein and might therefore be expected to be a dominant, gain-of-function mutation. Yet *ssu72-1* exacerbates the TFIIB defect, causing start site selection to shift further downstream of the normal site and creating a synthetic ts^- phenotype. Furthermore, *ssu72-1* is fully recessive with respect to $SSU72^+$. This anomaly can be rationalized, however, if the 10-amino-acid duplication encoded by *ssu72-1* impairs protein stability. This seems to be the case, since $SUA7^+$ *ssu72-1* mutants are mildly ts^-, albeit to a much lesser extent than *sua7-1 ssu72-1* double mutants (63) (Fig. 1, rows 2 and 3). The Cys_4 zinc binding motif might retain or even facilitate protein-protein interactions even though the 10-residue duplication destabilizes the protein. This could explain why the cysteine 25, which is not a component of the normal protein but is part of the duplication encoded by *ssu72-1*, is functionally critical (Table 2).

Start site selection in yeasts. In a yeast in vitro transcription system, replacement of both RNAP II and TFIIB from *S. cerevisiae* with their functional counterparts from *Schizosaccharomyces pombe* is sufficient to change the initiation pattern from that of *S. cerevisiae* to the pattern characteristic of *S. pombe* (38). On the basis of this result, Li et al. (38) concluded that RNAP II and TFIIB are the sole determinants of transcription start site selection in yeasts. This premise is consistent with the effects of altered forms of TFIIB (*sua7*), Rpb1p (*sua8*), and Rpb9p (*ssu73*) on start site selection (4, 20, 32, 50). In addition, the effects of *sua7* mutations on start site selection at the *CYC1* and *ADH1* genes are essentially identical to those of *sua8* mutations, and genetic evidence suggests that TFIIB interacts directly with the largest subunit of RNAP II (4). Clearly, RNAP II and TFIIB are critical determinants of start site selection, presumably as a consequence of specific interactions between Rpb1p, Rpb9p, and TFIIB.

What role does Ssu72p play in start site selection? It is important to recognize that our data do not indicate that *SSU72* necessarily plays a direct role in start site selection. Rather, an *ssu72* mutation enhances a TFIIB defect; the *ssu72-1* allele does not affect start site selection in an *SUA7* wild-type background (Fig. 6, lane 5). This result is similar to the effect of the *ssu71* suppressors. Mutations in *SSU71* restore the normal pattern of start site selection in an *sua7-1* background (64) yet have little or no effect in an $SUA7^+$ background (63). Among the *ssu71*, *ssu72*, and *ssu73* alleles, only *ssu73-1* (*RPB9*) influences start site selection in an $SUA7^+$ background (66). Therefore, our findings are consistent with the conclusion that start site selection is determined solely by interactions between TFIIB and RNAP II.

A possible role for Ssu72p in assembly of the transcription preinitiation complex. Ssu72p might facilitate assembly of the transcription preinitiation complex. This idea is based on the following rationale. Firstly, the E62K form of TFIIB encoded by *sua7-1* seems to impair assembly of TFIIB into the preinitiation complex. This is supported by the severe cs^- phenotype of *sua7-1* mutants, a phenotype often associated with defects in assembly of multisubunit complexes (reviewed in reference 73), and by the observation that TFIIB encoded by *sua7-1* is both functional and stable in vivo (51). Consequently, suppressors (*ssu71* and *ssu73*) or enhancers (*ssu72*) of the *sua7-1* mutation might include factors that affect assembly of the preinitiation complex. Secondly, the *ssu71* suppressors of *sua7-1* encode altered forms of the largest subunit of TFIIF and restore the normal pattern of transcription initiation in the *sua7-1* mutant (64). The Ssu71p subunit of TFIIF is homologous to mammalian RAP74. Although the RAP30 subunit of TFIIF is sufficient to initiate transcription (9) and to recruit RNAP II to the preinitiation complex (17, 34, 70), RAP74 seems to increase the efficiency of complex assembly (34, 70). These results suggest that RAP74 has a function in regulating assembly of the preinitiation complex (10). Since Ssu71p is homologous to RAP74 (64), Ssu71p might also function in preinitiation complex assembly. Thirdly, Ssu72p includes a half-site $C\text{-}X_2\text{-}C$ zinc binding motif that is critical for function and is duplicated in the *ssu72-1* mutant. Zinc binding domains within RNAP II have been proposed to stabilize subunit-subunit interactions, thereby facilitating assembly of the enzyme complex (69; reviewed in reference 3). Furthermore, the zinc binding motif of TFIIB is necessary to recruit RNAP II into the preinitiation complex (8), and the E62K defect (*sua7-1*) is located just downstream of this motif (51). Finally, the third effector of *sua7-1* uncovered in our studies, *ssu73-1*, encodes an altered form of Rpb9p. This RNAP II subunit includes two zinc binding motifs and has also been proposed to affect assembly of the RNAP II complex (3). Taken together, these results suggest that accurate start site selection involves specific interactions between RNAP II and TFIIB and that these interactions are mediated, at least in part, by zinc binding domains. The half-site zinc binding domain of Ssu72p might facilitate these interactions, perhaps as a component of the preinitiation complex that has gone undetected biochemically or as a chaperone-like protein that facilitates complex assembly.

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