MOLECULAR AND CELLULAR BIOLOGY, Jan. 1992, p. 248–260
0270-7306/92/010248-13$02.00/0

Vol. 12, No. 1

The *sui1* Suppressor Locus in *Saccharomyces cerevisiae* Encodes a Translation Factor That Functions during $tRNA_i^{Met}$ Recognition of the Start Codon

HEEJEONG YOON AND THOMAS F. DONAHUE*

Department of Biology, Indiana University, Bloomington, Indiana 47405

Received 23 July 1991/Accepted 9 October 1991

We initiated a genetic reversion analysis at the *HIS4* locus to identify components of the translation initiation complex that are important for ribosomal recognition of an initiator codon. Three unlinked suppressor loci, *sui1*, *sui2*, and *SUI3*, that restore expression of both *HIS4* and *HIS4-lacZ* in the absence of an AUG initiator codon were identified. In previous studies, it was demonstrated that the *sui2* and *SUI3* genes encode mutated forms of the α and β subunits, respectively, of eukaryotic translation initiation factor 2 (eIF-2). In this report, we describe the molecular and biochemical characterizations of the *sui1* suppressor locus. The DNA sequence of the $SUI1^+$ gene shows that it encodes a protein of 108 amino acids with a calculated M_r of 12,300. The *sui1* suppressor genes all contain single base pair changes that alter a single amino acid within this 108-amino-acid sequence. *sui1* suppressor strains that are temperature sensitive for growth on enriched medium have altered polysome profiles at the restrictive temperature typical of those caused by alteration of a protein that functions during the translation initiation process. Gene disruption experiments showed that the $SUI1^+$ gene encodes an essential protein, and antibodies directed against the $SUI1^+$ coding region identified a protein with the predicted M_r in a ribosomal salt wash fraction. As observed for *sui2* and *SUI3* suppression events, protein sequence analysis of His4–β-galactosidase fusion proteins produced by *sui1* suppression events indicated that a UUG codon is used as the site of translation initiation in the absence of an AUG start codon in *HIS4*. Changing the penultimate proline codon 3′ to UUG at *his4* to a Phe codon (UUC) blocks aminopeptidase cleavage of the amino-terminal amino acid of the His4–β-galactosidase protein, as noted by the appearance of Met in the first cycle of the Edman degradation reaction. The appearance of Met in the first cycle, as noted, in either a *sui1* or a *SUI3* suppressor strain showed that the mechanism of suppression is the same for both suppressor genes and allows the initiator tRNA to mismatch base pair with the UUG codon. This suggests that the Sui1 gene product performs a function similar to that of the β subunit of eIF-2 as encoded by the *SUI3* gene. However, the Sui1 gene product does not appear to be a required subunit of eIF-2 on the basis of purification schemes designed to identify the GTP-dependent binding activity of eIF-2 for the initiator tRNA. In addition, suppressor mutations in the *sui1* gene, in contrast to suppressor mutations in the *sui2* or *SUI3* gene, do not alter the GTP-dependent binding activity of eIF-2. The simplest interpretation of these studies is that the *sui1* suppressor gene defines an additional factor that functions in concert with eIF-2 to enable $tRNA_i^{Met}$ to establish ribosomal recognition of an AUG initiator codon.

The ribosomal scanning model has been proposed to account for fundamental features of eukaryotic mRNAs and basic steps during the pathway that leads to initiation of protein synthesis (17, 18). Briefly, eukaryotic translation initiation factor 2 (eIF-2), which is composed of three protein subunits, α, β, and γ, binds $tRNA_i^{Met}$ in the presence of GTP to form a ternary complex (15). This ternary complex, eIF-2–GTP–$tRNA_i^{Met}$, associates with the 40S ribosomal subunit, which in turn binds the 5′ end of the message and migrates in the 5′-to-3′ direction until the first AUG codon is found, where initiation occurs. The basic feature of this model has been corroborated in the yeast *Saccharomyces cerevisiae* by mutational studies of the *CYC1* (30) and *HIS4* (6, 9) genes. These studies suggest that by virtue of its proximity to the 5′ end of an mRNA, an AUG codon is used as the site of initiation. Therefore, the start site selection mechanism proposed for eukaryotes places the AUG codon and its interaction with the initiator $tRNA_i^{Met}$ as a fundamental event in this process. In fact, it has been previously demonstrated in our laboratory that ribosomal recognition of a start codon is mediated by the initiator tRNA (5). By introducing a mutation in the anticodon of $tRNA_i^{Met}$, it was shown that a mutant $tRNA_i^{Met}$ was capable of directing the ribosome to initiate translation at the first cognate codon in the *his4* message through a complementary anticodon-codon base pair interaction. However, considering the complexity of the eukaryotic translation initiation process, as reflected by the fact that eukaryotic initiation factors are composed of at least 25 proteins (24), it was conceivable that, in addition to $tRNA_i^{Met}$, other components of the translation initiation complex play an essential role in the start site selection process. Unfortunately, biochemical assays for specific steps during ribosomal recognition of a start codon have not been effective in identifying those factors that function in establishing the start site of translation.

To identify other components of the initiation complex that are important in defining start site selection in eukaryotes, we initiated a genetic analysis of the translation initiation process in *S. cerevisiae* (4). It has been demonstrated previously that mutations of the AUG start codon at *HIS4* leads to ribosomal bypass of the early *HIS4* coding region, resulting in a His^- phenotype (9). Therefore, by selecting for spontaneous His^+ revertants of these initiator codon mutant

* Corresponding author.

strains, mutations that no longer allow the ribosome to bypass this region were obtained (4). One type of His^+ revertant restored the AUG start codon at *HIS4*. However, by maintaining a second copy of the *his4* initiator codon mutation as part of a *his4-lacZ* gene fusion, extragenic suppressors were isolated on the basis of the ability to allow translation initiation to occur from both copies of the mutant messages, as noted by His^+ revertants that were blue on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside indicator plates. Three suppressor genes were identified by this selection method (4), i.e., *sui1*, *sui2*, and *SUI3* (suppressor of initiator codon mutations). The basic assumption was that these suppressor loci contain mutations in genes that encode *trans*-acting factors that alter, and therefore function in mediating, ribosomal recognition of the initiator region during the scanning process. That this assumption is correct was borne out by the molecular and biochemical analysis of the *sui2* (7) and *SUI3* (10) suppressor genes. The *sui2* suppressor gene encodes the α subunit of eIF-2, which is 58% identical to the human α subunit of eIF-2 (7). Mutations in the *sui2* suppressor genes that restore *his4* expression change amino acids that are identical at the same position in the human eIF-2 sequence (7, 12). The *SUI3* suppressor gene encodes the β subunit of eIF-2 (10) and is 42% identical to the human eIF-2β subunit (21). Mutations that confer *SUI3* suppression map to a putative zinc finger motif that is also conserved in the carboxyl region of the human β subunit (10). Both the *sui2* and the *SUI3* genes are essential for cell viability (7, 10). Amino-terminal sequence analysis of the His4–β-galactosidase protein produced as a result of either *sui2* or *SUI3* suppression events showed that in the absence of an AUG codon at *his4*, ribosomes no longer bypassed the mutated initiator region but recognized and initiated at a UUG codon in the early His4-coding region. These studies suggested that in addition to its initiator tRNA binding activity, eIF-2 was also involved in mediating the general mechanism of start site selection in all eukaryotic organisms.

In this report, we present the characterization of the *sui1* suppressor gene. Our analysis showed that the *sui1* gene encodes a previously unidentified 12.3-kDa protein. Described properties of *sui1* mutant strains and the Sui1 protein are consistent with the notion that this gene product performs an essential function during the translation initiation process in *S. cerevisiae*. In addition, we demonstrated that the effect of a suppressor mutation in the *sui1* gene is similar to that of a suppressor mutation in the β subunit of eIF-2 as encoded by *SUI3* and is to allow the normal translation initiation apparatus to initiate at a UUG codon in the early His4-coding region. Despite similar mechanisms of suppression, the Sui1 protein is not a subunit of eIF-2 that is required for ternary complex formation. We suggest that *sui1* encodes a translation initiation factor that functions with eIF-2 and the initiator tRNA in directing the ribosome to the proper start site of translation.

MATERIALS AND METHODS

Yeast strains and genetic methods. The yeast strains used in this analysis and their complete genotypes are listed in Table 1. All strains are related to TD28, an ascospore derivative of *S. cerevisiae* S288C (*MAT***a**). The standard genetic techniques and media used for these studies have already been described (29).

sui1 suppressor strains 117-8AR4 (*sui1-1*), 302-2D (*sui1-4*), 139-3C (*sui1-7*), and 138-7B (*sui1-17*) were used for molecular characterization of the *sui1* gene. The genetic identification and characterization of the *sui1* suppressor strains have been previously reported (4). Aside from the suppressor phenotype, restoration of *HIS4* expression (growth on synthetic dextrose medium lacking histidine [SD-histidine]) to yeast strains containing an initiator codon mutation at the *HIS4* gene, the *sui1* suppressor strains have a temperature sensitivity (Ts^-) phenotype for growth at 37°C on YEPD medium (yeast extract, peptone, dextrose). The suppressor phenotype and the Ts^- growth phenotype associated with *sui1* suppressor strains cosegregate in crosses (4).

TABLE 1. Yeast strains

Strain	Genotype
TD28	*MAT***a** *ura3-52 inol-13*
1172-3B	*MAT***a** *his4-316 ura3-52*
BC-64	*MAT*α *his4-401 ura3-52 leu2-3,-112*
117-8A	*MAT*α *his4-303*(AUU) *ura3-52*::*his4*(AUU)-*lacZ*(Ura^+)
EKP84	*MAT***a**/*MAT*α *ura3-52/ura3-52 LEU2/leu2-3,-112 HIS4*$^+$/*his4-306*(UUG)
117-8AR4	*MAT***a** *sui1-1 his4-303*(AUU) *ura3-52*::*his4*(AUU)-*lacZ*(Ura^+)
301-4D	*MAT***a** *sui1-1 his4-303*(AUU) *ura3-52 leu2-3,-112*
HY32	*MAT***a** *sui1-1 his4-303*(AUU) *ura3-52 leu2-3,-112 his4*(AUU/Phe)-*lacZ*(Ura^+)
302-2D	*MAT*α *sui1-4 his4-303*(AUU) *ura3-52 leu2-3,-112 inol-13*
139-3C	*MAT*α *sui1-7 his4-303*(AUU) *ura3-52*
138-7B	*MAT***a** *sui1-17 his4-301*(ACG) *ura3-52*
117-8AR20	*MAT*α *sui2-1 his4-303*(AUU) *ura3-52*::*his4*(AUU)-*lacZ*(Ura^+)
2119-11D	*MAT*α *SUI3-3 his4-303*(AUU) *ura3-52 leu2-3, -112 inol-13*
HY40	*MAT***a** *SUI3-3 his4-303*(AUU) *ura3-52 leu2-3, -112 inol-13 his4*(AUU/Phe)-*lacZ*(Ura^+)

Isolation and characterization of *SUI1* alleles. The wild-type *SUI1* gene was cloned from both YEp24 and YCp50 wild-type genomic clone banks. Positive clones were identified on the basis of complementation of the recessive Ts^- phenotype with a concomitant reduction or loss of the His^+ phenotype associated with *sui1* suppressor strains 302-2D, 139-3C, and 138-7B. Restriction analysis of the clones derived from the YEp24 and YCp50 banks indicated that the inserts were related and derived from the same chromosomal location.

The relationship of the cloned DNA fragment to the *SUI1* locus was established by genetic analysis. An 8.2-kb *Bam*HI DNA fragment from the YEp24 clone bank was subcloned into the *Bam*HI site of integrating yeast vector YIp5 and used to transform yeast strain TD28 to *URA3*$^+$. Ura^+ transformants were then crossed to *ura3-52 sui1* suppressor strains 139-3C and 302-2D and analyzed by tetrad analysis.

An approximately 10-kb *Sau*3A DNA insert was obtained from the YCp50 genomic bank, and this clone was used for subsequent characterization of the wild-type *SUI1* gene (see Fig. 1). Subcloning experiments for localization of the *SUI1* gene were performed with single-copy *URA3* yeast vector YCp50. The *SUI1* gene was localized to a 1.2-kb *Bgl*II-*Hin*dIII DNA fragment (see Fig. 1). The complete DNA sequence of this fragment (see Fig. 2) was determined for both strands by either the Maxam-and-Gilbert (19) or the chain termination DNA sequencing method (25).

A YCp50 plasmid, p1199, containing the wild-type *SUI1* gene as part of a 5.4-kb *Bgl*II-*Sal*1 DNA fragment (see Fig. 1; constructed from a partial *Bgl*II digest) was used to isolate

the *sui1-1*, *sui1-4*, *sui1-7*, and *sui1-17* suppressor alleles by the gap duplex repair method (20). For these experiments, plasmid p1199 was restricted at the *Bam*HI restriction site located within the coding region of the *SUI1* gene (see Fig. 1). Ts$^-$ *sui1* suppressor strains 301-4D, 302-2D, 139-3C, and 138-7B were transformed with this linearized DNA selecting for Ura$^+$ transformants. Ura$^+$ transformants were replica plated and tested for a temperature-sensitive growth phenotype on enriched medium and the ability to grow on SD-histidine plates. Ura$^+$ Ts$^+$ transformants that showed reduced growth on SD-histidine plates indicated that the wild-type gene was still present on the plasmid. Ura$^+$ Ts$^-$ transformants that maintained the His$^+$ suppressor phenotype indicated plasmids containing the corresponding *sui1* mutant allele as a result of repair-associated gene conversion events. Plasmids from the Ts$^-$ His$^+$ transformants were isolated in *Escherichia coli*, and the presence of a single mutation in the coding region of each of the *sui1* suppressor alleles was confirmed by DNA sequencing.

Analysis of *SUI1* expression. For gene disruption experiments (23), a 2.3-kb *Xho*I-*Hin*dIII DNA fragment (see Fig. 1) containing the intact *SUI1* gene was ligated into the *Sal*I and *Hin*dIII sites of pBR322 to yield plasmid p1134. A 1.1-kb *Hin*dIII fragment containing the *URA3* gene was adapted with *Bam*HI ends and inserted into the *Bam*HI site within the *SUI1* coding region (see Fig. 2) of p1134 to construct plasmid p1209 (*SUI1*::*URA3*$^+$). As a result of this construction, the *SUI1* coding region was disrupted. p1209 was restricted with either *Sph*I or *Cla*I and *Kpn*I and used to transform diploid strain EKP84 to Ura$^+$. Restriction of p1209 with these enzymes generated an approximately 3.4-kb *SUI1*::*URA3*$^+$ DNA fragment. Transformants were analyzed by tetrad analysis. Genomic DNA was isolated from one of these diploid Ura3$^+$ transformants, HJY3, and from *SUI1* wild-type haploid strain 117-8A. The genomic DNAs were restricted with *Sac*II and *Bgl*II (see Fig. 1) and analyzed by Southern blotting.

The 1,200-bp *Bgl*II-*Hin*dIII DNA fragment (see Fig. 1) containing the wild-type *SUI1* gene was subcloned into the *Bam*HI and *Hin*dIII sites of YCp50 to produce plasmid p1128. p1128 was then restricted with *Bam*HI in the *SUI1* coding region (see Fig. 1), and self-complementary oligonucleotide 5′-GATCTCTAGA-3′ was ligated into the *Bam*HI site. Insertion of this oligonucleotide resulted in a +1 frameshift mutation in the *SUI1* coding region and a subsequent in-frame translation termination signal, TAG, as part of the oligonucleotide. This plasmid, p1511, was used to transform *sui1* suppressor strains 138-7B, 139-3C, 301-4D, and 302-2D to Ura$^+$. Transformants were tested for the ability to complement the Ts$^-$ phenotype associated with these *sui1* suppressor strains.

For Northern (RNA) analysis, total and poly(A)$^+$ RNAs were isolated from wild-type and *sui1* mutant strains as previously described (6). A 50 μg sample of total RNA or 10 μg of poly(A)$^+$ RNA was electrophoresed on a 6% acrylamide–8 M urea gel (3). Gels were electroblotted to nylon filters in 1 mM Tris-acetate (pH 7.8)–5 mM sodium acetate–0.5 mM EDTA at 20 V overnight. Blots were fixed by germicidal lamp exposure (330 μW/cm^2 for 2 min). The conditions for prehybridization and hybridization have already been reported (4). The 5′ map position of the *SUI1*$^+$ transcripts was determined by primer extension as previously described (6), by using 50 μg of total RNA isolated from yeast strain 1172-3B and the oligonucleotide 5′-TTCG TCGTCTCCTGTGTCGGCGAAAGGATC-3′, which is complementary to nucleotide positions +28 to +57 in the *SUI1* coding region (see Fig. 2).

For both Southern and Northern analyses, a 1.4-kb *Sph*I restriction fragment containing the entire *SUI1* gene was used as a probe. ^{32}P-labelled DNA was prepared by the random-primer labelling method.

The proximal *Bgl*II-*Bam*HI DNA fragment from the *SUI1* wild-type gene (see Fig. 1) was inserted into the *Bam*HI site of plasmid p349 (9), which contains the *E. coli lacZ* coding region as part of YCp50. This plasmid, p1132, results in an in-frame *sui1-lacZ* fusion and was used to transform yeast strain TD28 to *URA3*$^+$. Transformants were screened on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside indicator plates, and the β-galactosidase activities of transformants were determined as previously described (9).

Site-directed mutations at the *HIS4* gene. Mutations in the *HIS4* coding region were constructed by site-directed mutagenesis as previously described (9). The template used was bacteriophage vector M13mp11, containing a 1,578-bp *Sal*I DNA insert from the proximal region of the *HIS4* gene (11). The oligonucleotide used to introduce mutations in the *HIS4* coding region was 5′-TGAATAATTGTTTTGTTCATTCTA CC-3′. This oligonucleotide changed the *HIS4* AUG start codon to AUU and amino acid position 4 in the *HIS4* coding region from a proline (CCG) to a phenylalanine (UUC) codon. Positive plaques for each construct were identified by hybridization with the corresponding ^{32}P-labelled oligonucleotide, and the presence of the mutations in the *HIS4* region was confirmed by DNA sequencing. A 763-bp *Sau*3A fragment which contains the full, intact *HIS4* promoter region and the first 11 amino acids of the *HIS4* coding region was then subcloned from two independent constructs into the *Bam*HI site of p349 to yield an in-frame *his4-lacZ* fusion plasmid. We refer to this mutated *his4-lacZ* plasmid construct as *his4*(AUU/Phe)-*lacZ*. This plasmid was used to transform yeast strains 301-4D (*sui1-1*) and 2119-11D (*SUI3-3*) to Ura$^+$, which contain a deletion of the *HIS4* gene. Transformants were then nonselectively screened on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates for blue colonies. His$^+$ blue colonies from each transformation were used for purification and amino-terminal sequence analysis of the His4–β-galactosidase protein produced as a result of suppression events (see below). The corresponding yeast strains used for His4–β-galactosidase sequence analysis are referred to as HY32 [*sui1-1 his4*(AUU/Phe)-*lacZ*] and HY40 [*SUI3-3 his4*(AUU/Phe)-*lacZ*].

Polysome analysis. Polysome profiles of Ts$^-$ *sui1* mutants 117-8AR4 and 138-7B were analyzed from cultures incubated under permissive (23°C) and restrictive (37°C) growth conditions and compared with the polysome profiles of the parent *SUI1*$^+$ strain, 117-8A, and a Ts$^-$ *sui2* mutant strain, 117-8AR20, incubated at the same temperatures. Two cultures of each strain (200 ml) were grown overnight at 23°C to an optical density at 600 nm (OD_{600}) of 1.0 in liquid YEPD medium. One of the two cultures from each strain was then shifted to 37°C and grown for two more hours. At the end of the 2-h growth period, cycloheximide (10 mg) was added to each of the cultures grown at 23 and 37°C and extracts were prepared as described by Baim et al. (2). Fifty OD_{260} units of each extract was layered on top of a 35-ml linear sucrose gradient (7 to 47% [wt/vol]) containing 50 mM Tris-acetate (pH 7.0), 50 mM NH_4Cl, 12 mM $MgCl_2$, 1 mM dithiothreitol, 0.1% diethy pyrocabonate (DEPC), and 1 mM phenylmethylsulfonyl fluoride. The gradient was prepared by underlaying in a stepwise fashion 7 ml each of 17, 27, 37, and 47% sucrose below an initial 7 ml of 7% sucrose in a centrifuge

tube and allowed to equilibrate overnight at 4°C. Sucrose gradients were centrifuged at 24,000 rpm in an SW27 rotor for 4.5 h. Gradients were collected from the top by using an ISCO 640 density gradient fractionator and monitored by the ISCO UA-5 absorbance-fluorescence detector. The 40S and 60S peaks of the polysome gradients were determined by Northern analysis. rDNA probes were prepared by labelling *Eco*RI fragments restricted from pBR322-derived plasmids pBR322C and pBR322A, which contain 18S (40S) and 25S (60S) rDNAs, respectively (22).

Immunological methods. Antibodies directed against the *SUI1* gene product were generated from TrpE-Sui1 fusion proteins expressed in *E. coli*. The distal *SUI1* coding region from amino acid positions +71 to +108 contained on a 445-bp *Bam*HI-*Hin*dIII DNA fragment (see Fig. 1) was ligated in frame to the carboxyl end of the *trpE* coding region as part of the pATH2 expression vector (8). A second construct was made by ligating the 496-bp *Eco*RI-*Hin*dIII DNA fragment (see Fig. 1) from the distal end of *SUI1* to pATH1, which results in an in-frame fusion of TrpE to amino acid positions +54 to +108 of the *SUI1* coding region. The methods employed for extraction of the antigen and immunizations have already been reported (10). The specificities and titers of the antisera were determined by Western blot (immunoblot) analysis by using extracts prepared from single-copy *SUI1* strain BC64 and isogenic strain BC61, which is identical to BC64 but contains the *SUI1* gene on high-copy *URA3*$^+$ vector YEp24. Preparation of cell extracts and the Western blot conditions used have been previously described (10).

Fractionation and assay of eIF-2. eIF-2 was partially purified from *SUI1* wild-type strain 117-8A and *sui1* suppressor strains 117-8AR4 and 138-7B by a modification of the method previously described for eIF-2 purification from *S. cerevisiae* (10). A 12-liters volume of cells was grown at 23°C to an OD_{600} of 1.3. Cells were harvested and washed twice in double-distilled H_2O. Cells were suspended in a lysis buffer containing 20 mM Tris-HCl (pH 7.5), 100 mM KCl, and 30 mM $MgSO_4$ and then broken with glass beads in a Bead Beater. Extracts were cleared by two sequential centrifugations (5,000 × *g* for 10 min and 22,000 × *g* for 30 min), and ribosomes were isolated by centrifugation at 200,000 × *g* for 2 h. Ribosomal pellets were washed in lysis buffer containing 500 mM KCl and recentrifuged for 2 h at 200,000 × *g*. The supernatant was then dialyzed overnight against 20 mM Tris-HCl (pH 7.5)–100 mM KCl. This ribosomal salt wash was then fractionated in a stepwise fashion with a saturated solution of ammonium sulfate. The 55 to 60% ammonium sulfate pellet was then suspended in 20 mM KPO_4 (pH 7.5) containing 5% glycerol (buffer A) and dialyzed against the same buffer overnight. The dialyzed sample was loaded onto a hydroxyapatite (HAP) column (1.2 by 3.3 cm) that was pre-equilibrated with buffer A. After unbound proteins were washed from the column with buffer A, eIF-2 was eluted by using a 100 to 600 mM linear gradient of KPO_4 (pH 7.5) containing 5% glycerol. Fractions (2 ml) were concentrated by using a Centricon 10 and assayed for eIF-2 activity. All eIF-2 purification steps were performed at 4°C, and the buffers used for fractionation contained the protease inhibitors 1 mM phenylmethylsulfonyl fluoride and 0.7 μg of pepstatin A per ml. Protein concentrations were determined by the dye-binding method of Bradford (Bio-Rad Laboratories) with bovine serum albumin as the standard. eIF-2 activity was determined as previously described (10), except for a change in GTP concentration being reduced in the assay mixture from 1.2 to 0.12 mM.

Protein sequence analysis. His4–β-galactosidase protein was purified from yeast strains as previously described (10). For some experiments, the His4–β-galactosidase protein was purified by a modification of the latter part of the published procedure. In these experiments, His4–β-galactosidase was eluted from the *p*-aminobenzyl-1-thio-β-D-galactopyranoside–agarose column and peak activity fractions were concentrated and dialyzed with a Centricon 30. Protein concentrations were determined, and aliquots were run on a sodium dodecyl sulfate (SDS)–7.5% polyacrylamide gel that contained 10 μM sodium thioglycolate in the electrophoresis buffer in the upper reservoir. The gel was blotted to a ProBlot nylon membrane. The transfer buffer contained 10 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid] (pH 11.0) with 10% methanol. Blotting was performed at 300 mA for 1 h, and the membrane was stained with Coomassie blue to visualize the His4–β-galactosidase protein. Purified His4–β-galactosidase (M_r, approximately 116,000) was identified as a single protein band that comigrated on gels with *E. coli* β-galactosidase (M_r, approximately 115,000) which was used as a molecular weight standard for each experiment. The His4–β-galactosidase band identified on the filter was then excised and used for amino-terminal sequence analysis. β-Galactosidase specific activities of crude extracts were 4 U for parent strain 117-8A, 22 U for *sui1-1* suppressor strain 117-8AR4 [*his4*(AUU)-*lacZ*], 450 U for *HIS4*$^+$-*lacZ SUI1*$^+$ wild-type strains, 18 U for HY32 (*sui1-1* AUU/*Phe*), and 150 U for HY40 (*SUI3-3* AUU/Phe). The amino-terminal sequence of His4–β-galactosidase protein was determined at the Northwestern University Biotechnology Center by automated Edman degradation using an Applied Biosystems (Foster City, Calif.) 120A on-line phenylthiohydantoin amino acid analyzer (16). Approximately 10 to 100 pmol of His4–β-galactosidase was estimated to have been used for the different analyses.

Nucleotide sequence accession number. The nucleotide sequence presented here has been assigned GenBank accession no. M77514.

RESULTS

Characterization of the *SUI1*$^+$ gene. The wild-type *SUI1* gene was identified as part of approximately 9- and 10-kb DNA inserts from YEp24 and YCp50 wild-type genomic clone banks, respectively, on the basis of its ability to complement the recessive Ts$^-$ phenotype and the poor growth characteristics at 23°C associated with *sui1* mutant strains. In addition, these clones, when present in *sui1* strains, either reduced or abolished the His4$^+$ suppressor phenotype, indicating that, as observed in *sui1*/*SUI1*$^+$ diploids, the wild-type gene acted in either a codominant or a dominant fashion, depending on the *sui1* allele (4). (We indicate by genetic nomenclature that the *sui1* suppressor alleles are recessive to the wild-type allele [4]. Although the Ts$^-$ phenotype is recessive, it should be noted that the suppressor phenotype associated with different *sui1* strains behaves as either a recessive or a very weak codominant trait in *sui1*/*SUI1*$^+$ heterozygotes. We believe this is due to an altered function of low efficiency that gives rise to *sui1* suppression events [4] and not a reflection of a trait that is truly recessive. Similar observations have also been made with the *sui2* suppressor locus, which encodes altered forms of the α subunit of eIF-2 [4].) An 8.2-kb *Bam*HI fragment derived from the YEp24 bank was subcloned into the integrating YIp5 vector and used to transform *SUI1* wild-type yeast strain TD28 to Ura$^+$. Transformants were purified and

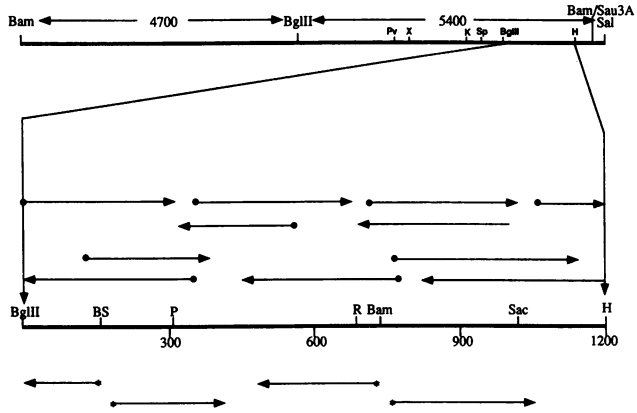

FIG. 1. Restriction map and sequencing strategy for the wild-type *SUI1* clone. The approximately 10.1-kb *Bam*HI–*Bam*HI-*Sau*3A DNA fragment was identified from a YCp50 clone bank on the basis of the ability to complement the temperature-sensitivity phenotype associated with *sui1* suppressor strains. The *SUI1* gene was localized to the 1,200-bp *Bgl*II-*Hin*dIII DNA fragment. The direction and extent of DNA sequencing by the dideoxy (25) and Maxam-and-Gilbert (19) methods are indicated as arrows with closed circles and asterisks, respectively. Restriction site abbreviations: Bam, *Bam*HI; BS, *Bss*HII; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; R, *Eco*RI; Sac, *Sac*II; Sal, *Sal*I; Sau3A, *Sau*3AI; Sp, *Sph*I; X, *Xho*I.

crossed to Ts$^-$ Ura3$^-$ *sui1* suppressor strains. Among 21 tetrads analyzed, all Ura$^+$ ascospores were Ts$^+$ whereas all Ura$^-$ ascospores were Ts$^-$, indicating that the DNA region was derived from the *SUI1* locus. The ability to complement the *sui1* Ts$^-$ phenotype was localized to a 1.2-kb *Bgl*II-*Hin*dIII DNA fragment (Fig. 1). Southern analysis showed that this region is unique in the haploid genome of *S. cerevisiae* (data not shown).

The DNA sequence of the 1.2-kb *Bgl*II-*Hin*dIII region is shown in Fig. 2. An open reading frame of 108 amino acids that begins with an ATG is contained in this sequence and would encode a protein with a calculated M_r of 12,300. Northern analysis of total and poly(A)$^+$ RNAs isolated from wild-type and mutant *sui1* strains identified a unique transcript from this region. On the basis of estimation of an R_f value, this transcript is between 400 and 600 nucleotides long, which is sufficient to encode a 108-amino-acid protein (data not shown). Primer extension analysis (data not shown) identified major and minor start positions for the transcription of this region with two major transcripts having 5′ ends that mapped at base pair positions −18 and −20 upstream from the first ATG that defines the 108-amino-acid opening reading frame (Fig. 2).

To demonstrate that the 108-amino-acid open reading frame contained on the *Bgl*II-*Hin*dIII *SUI1* clone (p1128) is responsible for complementation of the Ts$^-$ and His$^+$ suppressor phenotype of *sui1* mutant strains, plasmid p1511 was constructed to contain a +1 frameshift mutation after amino

```
                    BglII
                    agatctgaatctattctggacatcctgttctagctgctagtactatcactactactgctatggctgtattactaata

                                                                                          BssHII
tttctattgtttcactattctttatatatatttatatatgtatattttccagcaaaacacccttttggcattctcctataggacgtcaaacggcgcgcg

accgcgtcattttatacgagcagccgctgcgtcgtcgtacaaaacgcaccgacctacattacaataaaaagtacccgtaaaaggatcatgtgtacatggt

                                  PstI
tccagtcttttggttcggtactataactactgtaaacgacccctctcactgcagtttatgatatgtgatcgcgttaaaaatgggtattatcaccaataacg

ggtcgggtaacagatttttcgatgcgccggcaaaaaatttagttttttttttttttcatcacagggataaaagcaaactgcaaaaggacaaaacaagag

aagagctacttgttgttttcgtttcgtgctgggggttttctcctttctattctttttcaaaaaagacaaggaatatattaatatagctgaagcaaatcgt

1
ATG TCC ATT GAG AAT CTG AAA TCA TTT GAT CCT TTC GCC GAC ACA GGA GAC GAC GAA ACC GCC ACT TCA AAC TAT
Met Ser Ile Glu Asn Leu Lys Ser Phe Asp Pro Phe Ala Asp Thr Gly Asp Asp Glu Thr Ala Thr Ser Asn Tyr

26
ATT CAT ATT CGT ATC CAA CAG AGA AAT GGT AGA AAA ACT TTA ACT ACG GTG CAA GGT GTC CCA GAG GAA TAT GAT
Ile His Ile Arg Ile Gln Gln Arg Asn Gly Arg Lys Thr Leu Thr Thr Val Gln Gly Val Pro Glu Glu Tyr Asp

51      EcoRI                                                                    BamHI
TTA AAG AGA ATT CTT AAG GTC CTA AAG AAG GAC TTT GCA TGT AAT GGT AAC ATT GTC AAG GAT CCA GAA ATG GGG
Leu Lys Arg Ile Leu Lys Val Leu Lys Lys Asp Phe Ala Cys Asn Gly Asn Ile Val Lys Asp Pro Glu Met Gly

76                        **   *
GAG ATT ATT CAG TTG CAG GGT GAC CAA AGA GCA AAG GTT TGC GAA TTT ATG ATC TCC CAA CTG GGA TTG CAA AAG
Glu Ile Ile Gln Leu Gln Gly Asp Gln Arg Ala Lys Val Cys Glu Phe Met Ile Ser Gln Leu Gly Leu Gln Lys

101
AAG AAC ATT AAA ATT CAT GGG TTT TAA gttcaaggcttacgccgagtcgcttactcgctgctcgctttctttcttagtgttgttatcctat
Lys Asn Ile Lys Ile His Gly Phe Ter

ataaaacgtaactgtttagttatttaagtccaagtgcatatttacactataatatataatataacatctcatctttttttatttctttttcttcttctcc

cgccgcgggcttacatacagagatataagaacaagtggtacgtagcatgcagcttctcaaaagagatgagcgttcagttttgagtacagggaagaaaggc

                                                    HindIII
caaatcgagagtacattatcatggggtcgggcgatttgaatctgttgaaatcatggaatcccaagctt
```

FIG. 2. DNA sequence analysis of the wild-type *SUI1* gene. The DNA sequence corresponding to the 108-amino-acid sequence of the *SUI1* gene product was confirmed by either the Maxam-and-Gilbert or dideoxy chain termination method (see Fig. 1 for sequencing strategy). The asterisks (left to right) indicate the nucleotides changed in the mutant *sui1-4* and *sui1-7* alleles (GAC [Asp] to TAC [Tyr]) and the *sui1-1* mutant allele (GAC [Asp] to GGC [Gly]) that altered amino acid position +83 and the *sui1-17* mutant allele (CAA [Gln] to CCA [Pro]) that altered amino acid position +84. The large arrowheads designate the major transcriptional start sites that mapped to basepair positions −20 and −18 (relative to the ΔTG; +1 position), and the small arrowheads indicate minor start sites that mapped to base pair positions −26, −24, and −14, as determined by primer extension analysis.

acid +71 in the open reading frame and used to transform *suil* suppressor strains. Transformants containing plasmid p1511 maintained the same Ts$^-$ and His$^+$ suppressor phenotypes as the parent *suil* suppressor strains. In contrast, the same strains transformed with *SUI1*$^+$ parent plasmid p1128 were Ts$^+$ and had either a reduced His$^+$ suppressor phenotype or were His$^-$, depending on the *suil* strain. This indicates that translation of this 108-amino-acid open reading frame results in ability of this DNA fragment to complement *suil* mutants.

To test the expression of this 108-amino-acid open reading frame in *S. cerevisiae*, we constructed an in-frame fusion of the first 71 amino acids (Fig. 2) to the *lacZ* coding region, as part of a 785-bp *Bgl*II-*Bam*HI DNA fragment (Fig. 1). This construct, when present in *S. cerevisiae*, produced a significant level of β-galactosidase (258 U), indicating that the 785-bp *Bgl*II-*Bam*HI DNA fragment was capable of directing the transcription and translation of this 108-amino-acid open reading frame. Immunoprecipitation of this fusion protein from cell extracts with antibody raised against β-galactosidase identified a protein with the M_r (120,000) predicted for the fusion protein. Gene disruption of this 108-amino-acid open reading frame in a wild-type diploid strain and subsequent genetic analysis of 77 tetrads from eight independent diploid transformants resulted in 53 tetrads showing a two viable-two inviable segregation ratio, while the remaining 24 tetrads had only one viable spore. All viable spores were Ura$^-$, indicating that disruption of the *SUI1* gene by the *URA3*$^+$ gene leads to cell inviability. Therefore, this 108-amino-acid-coding region is transcribed and translated in *S. cerevisiae* and encodes an essential gene product.

Characterization of the *suil* suppressor genes. *suil* suppressor mutations were mapped and isolated by the gap duplex repair method (20). Plasmid p1199 was used to transform Ts$^-$ *suil* suppressor strains 117-8AR4 (*suil-1*), 302-2D (*suil-4*), 139-3C (*suil-7*), and 138-7B (*suil-17*) to Ura3$^+$. This plasmid contains the intact 108-amino-acid open reading frame as part of a 5.4-kb DNA insert in YCp50. Restriction of this plasmid at a unique *Bam*HI restriction site prior to transformation yielded a higher frequency of Ts$^-$ transformants than either the uncut plasmid or the plasmid restricted at a unique *Pvu*II restriction site. This *Bam*HI site is within the 108-amino-acid open reading frame (Fig. 2), while the *Pvu*II site lies outside the open reading frame (Fig. 1). This higher incidence of Ts$^-$ transformants suggested that the site of the mutation that confers the Ts$^-$ phenotype was nearer the *Bam*HI site and incorporated into the corresponding DNA insert region of the plasmid during gap duplex repair of the restricted plasmid in *S. cerevisiae*.

Plasmids were isolated from these Ts$^-$ transformants, and the DNA sequence of the 108-amino-acid open reading frame was determined. As expected, the DNA sequence analysis identified a single-base change in each allele that altered one of the amino acids in the 108-amino-acid open reading frame (Fig. 2). The sequence from the *suil-1* strain contained an aspartic acid (GAC)-to-glycine (GGC) change at amino acid position +83. The *suil-4* and *suil-7* alleles both contained the identical base mutation that changed the same aspartic acid (+83) to a tyrosine (TAC). The sequence of the *suil-17* allele showed that amino acid position +84 had been changed from a glutamine (CAA) to a proline (CCA) codon.

Aside from gap duplex repair and DNA sequence analyses of the mutant *suil* alleles, other experiments supported the notion that a single base change in the 108-amino-acid-coding region was responsible for the Ts$^-$ and His$^+$ suppressor phenotypes of these *suil* mutants. (i) All *suil* suppressor mutants were identified as spontaneous His$^+$ revertants (4). (ii) The Ts$^-$ and His$^+$ suppressor phenotypes of *suil* mutants are unstable, and Ts$^+$ His$^-$ revertants are easily obtained. (iii) Integration of the large *Bam*HI DNA fragment from the wild-type *SUI1* clone (Fig. 1) as part of YIp5 at a number of *suil* suppressor alleles does not alter the Ts$^-$ and His$^+$ suppressor phenotypes of the *suil* suppressor mutant strains used for transformation. This latter observation indicates that each mutation that confers suppression maps either 3′ or very close to the *Bam*HI site in the 108-amino-acid-coding region of *suil* alleles. The basis for this conclusion is that the *Bam*HI subclone contains only the 5′ noncoding region and the first 71 amino acids of the wild-type 108-amino-acid-coding region (Fig. 2). Therefore, if substitution of this region by integrative transformation for the corresponding region in the *suil* alleles has no affect on the Ts$^-$ or His$^+$ phenotypes, then the mutation(s) that confers suppression is not contained in the 5′ noncoding region or the first 71 amino acids of the coding region. Thus, genetic data suggest that the Ts$^-$ and His$^+$ phenotypes associated with *suil* suppressor strains are a result of a single base change that maps 3′ to amino acid position +71 in the 108-amino-acid-coding region and agrees with our gap duplex and DNA sequence analyses of *suil* alleles (Fig. 2). In light of these and the above-described studies, we conclude that the 108-amino-acid open reading frame encodes the *SUI1* gene product.

***suil* Ts$^-$ mutants have an in vivo defect in translation initiation.** On the basis of the genetic selection scheme we employed (4) and the molecular analysis of the *suil* gene, it seemed logical to assume that the *suil* gene product performed an essential function during the translation initiation process in *S. cerevisiae*. In light of this assumption, one simple interpretation of the Ts$^-$ growth phenotype of *suil* strains is that at the restrictive temperature (37°C) the Sui1 protein is thermolabile. As a result of this thermolability, the Sui1 protein cannot perform its essential function during the translation initiation process that leads to the inhibition of protein synthesis, which results in inhibition of cell growth.

To test this possibility, duplicate cultures of a Ts$^-$ *suil* mutant were grown at 23°C and then one culture was shifted to 37°C for 2 h. Extracts were prepared from both of the cultures and fractionated by sucrose gradients, and polysome profiles were compared. Figure 3A shows the nearly identical polysome profiles from two extracts prepared from a *SUI1*$^+$ control strain, one from a culture which had been grown only at 23°C and the other extract derived from a culture that had been grown at 23°C and then shifted to 37°C for 2 h. In contrast, the polysome profile of an isogenic *suil* Ts$^-$ mutant grown in an identical fashion at 23°C was similar to that of the wild type but an aberrant profile was obtained from the extract which was derived from a culture that had been shifted to the restrictive temperature for 2 h (Fig. 3B). In essence, little remained of the larger polysomes at 37°C and a significant increase in the 80S region of the gradient was observed. (This 80S peak appears to represent an mRNA-independent association of 40S and 60S subunits.) The same polysome profile was also observed in an independent Ts$^-$ *suil* mutant (data not shown), as well as a Ts$^-$ *sui2* strain (Fig. 3C) which encodes a defective α subunit of eIF-2, a protein known to be essential for translation initiation in *S. cerevisiae* and other eukaryotic organisms. The simplest explanation for the similar polysome profiles observed in *suil* and *sui2* mutants is that the *suil* gene also encodes a protein that has an essential function during translation initiation. This product is thermolabile in the *suil* mutant strain, and therefore upon temperature shift it cannot

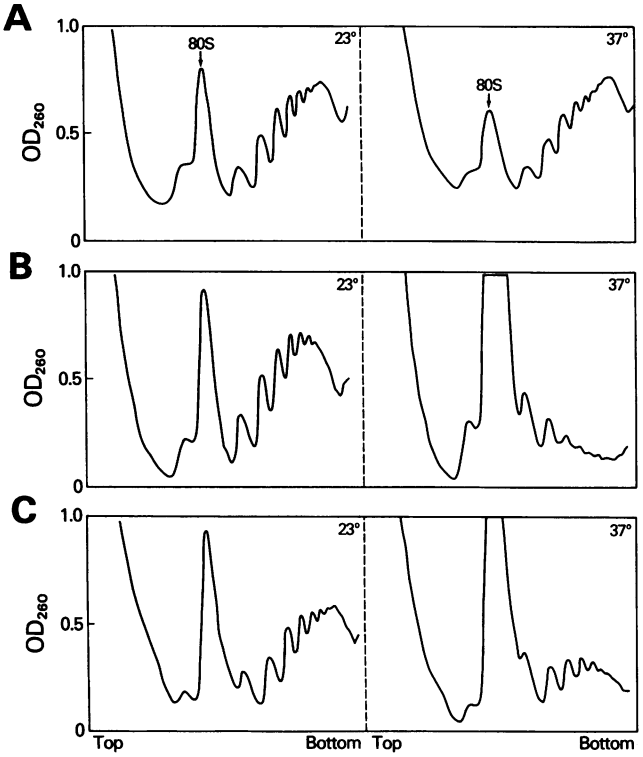

FIG. 3. Polysome analysis. Duplicate cultures of either a wild-type strain, a Ts⁻ *sui1* suppressor strain, or a Ts⁻ *sui2* suppressor strain were grown to an OD_{600} of 1.0 at 23°C. One of the duplicate cultures was then shifted to 37°C, and both the 23 and 37°C cultures were incubated for two more hours. Cells from each culture were then harvested, cell extracts were prepared, and 50 OD_{260} U from each extract was fractionated on a 7 to 47% sucrose gradient (35 ml) by centrifugation in an SW27 rotor at 24,000 rpm for 4.5 h. Gradients were collected from the top by using an ISCO 640 density gradient fractionator and monitored by the ISCO UA-5 absorbance-fluorescence detector. Gradients are plotted as OD_{260} units versus fraction (1.2 ml) numbers from the top to the bottom of the gradient. The position of 80S ribosomes in each gradient is marked and was determined by probing the various fractions by Northern analysis as described in Materials and Methods. (A) Polysome profiles prepared from *SUI1*⁺ wild-type parent strain 117-8A grown at 23°C (left) and 37°C (right). (B) Polysome profile of isogenic Ts⁻ *sui1* suppressor strain 117-8AR4 grown at 23°C (left) and 37°C (right). (C) Polysome profile of isogenic Ts⁻ *sui2* suppressor strain 117-8AR20 grown at 23°C (left) and 37°C (right).

perform its function. This leads to a block in translation initiation, which leads to accumulation of 40S and 60S subunits (14). However, translation elongation is not affected by the mutation in *sui1* and therefore polysomes finish translating mRNA during the temperature shift, which leads to a substantial reduction in the polysomes, as exhibited in each mutant profile.

Mechanism of *sui1* suppression. Previous studies in our laboratory demonstrated that the *sui2* and *SUI3* suppressor genes that encode mutant α and β subunits of eIF-2, respectively, restored translation initiation to *his4* and *his4-lacZ* initiator codon mutant strains by allowing initiation at a UUG codon present at amino acid position +3 in the early *his4* coding region (7, 10). To determine whether mutations in the *sui1* suppressor gene also restore His4 expression by a similar mechanism, the His4–β-galactosidase fusion protein produced from the mutant *his4-lacZ* mRNA in a *sui1-1* strain was purified and subjected to amino-terminal sequence analysis. The yields of phenylthiohydantoin amino acids from the first five cycles of the protein sequence when an AUU initiator codon is present in the *his4-lacZ* message are depicted as a bar graph in Fig. 4A. The predominant sequence, Pro-Ile-Leu-Pro-Leu, matches the *HIS4* coding region beginning at codon 4 in the message. These data parallel the sequence analysis of His4–β-galactosidase protein produced from an AUU *his4-lacZ* message as a result of either *sui2* or *SUI3* suppression (7, 10).

One interpretation of our amino-terminal sequence analysis of the His4–β-galactosidase protein produced from a mutant *his4-lacZ* message is that the standard methionine initiator tRNA is capable of initiating translation at a UUG codon as a result of the mutation in either a *sui1* (Fig. 4A), a *sui2* (7), or a *SUI3* (10) suppressor gene. The methionine inserted is then cleaved in vivo by an aminopeptidase and therefore does not appear in the first cycle of the sequence analysis. However, given the facts that the *sui2* and *SUI3* genes encode the α and β subunits of eIF-2 and eIF-2 is known to be involved in initiator tRNA binding, it is also plausible, mechanistically, that mutations in either *sui2* or *SUI3* alter the tRNA-binding activity of eIF-2. Perhaps the mutant forms of these proteins allow eIF-2 to bind a Leu tRNA and use it to initiate at a complementary UUG codon at amino acid position +3 in the early *his4* coding region. Again, leucine might not appear in the first cycle of the protein sequence analysis, as an aminopeptidase would remove it from the fusion protein in vivo. The significance of this distinction is that in the former case the *sui* suppressor genes would be involved in mediating ribosomal recognition of the start codon in conjunction with the initiator tRNA, whereas in the latter case the suppressor genes may function only in binding the initiator tRNA and the complementary anticodon-codon interaction would be the primary determinant in establishing start site selection.

To differentiate between these two different mechanistic interpretations of suppression, we sequenced the His4–β-galactosidase fusion proteins produced from a *sui1* strain and a *SUI3* strain that contained an AUU initiator codon mutation in the *his4-lacZ* message and an additional codon change at position +4 in the early coding region of *his4*. The codon change substituted a phenylalanine codon (TTC) for the normal proline codon (CCG) at amino acid position +4. The basic assumption of this codon alteration was that if methionine was inserted at UUG, then by increasing the Stokes radius of the penultimate amino acid next to the UUG codon the ability of aminopeptidases to cleave methionine would be inhibited (28). Therefore, we could differentiate between suppression events that allow methionine initiator tRNA to initiate translation at UUG from another tRNA species. If Met is inserted at UUG as a result of suppression, then the penultimate Phe blocks its cleavage by aminopeptidase. This would be noted by the appearance of Met as the predominant amino acid in the first cycle of the protein sequence.

As shown in Fig. 4B, when phenylalanine is present at amino acid position +4 in the *his4* coding region in a *sui1* strain, the amino acid sequence is drastically changed compared with an isogenic strain that contains only the AUU mutation at the *his4* initiator region (Fig. 4A). The predominant amino acid sequence obtained now reads Met-Phe-Ile-Leu-Pro (Fig. 4B). The identical sequence, Met-Phe-Ile-Leu-Pro, is also obtained for the purified His4–β-galactosidase protein produced in a *SUI3* suppressor strain (Fig. 4C) when a phenylalanine codon is present at the +4 amino acid position in the *his4* coding region of the same mutant

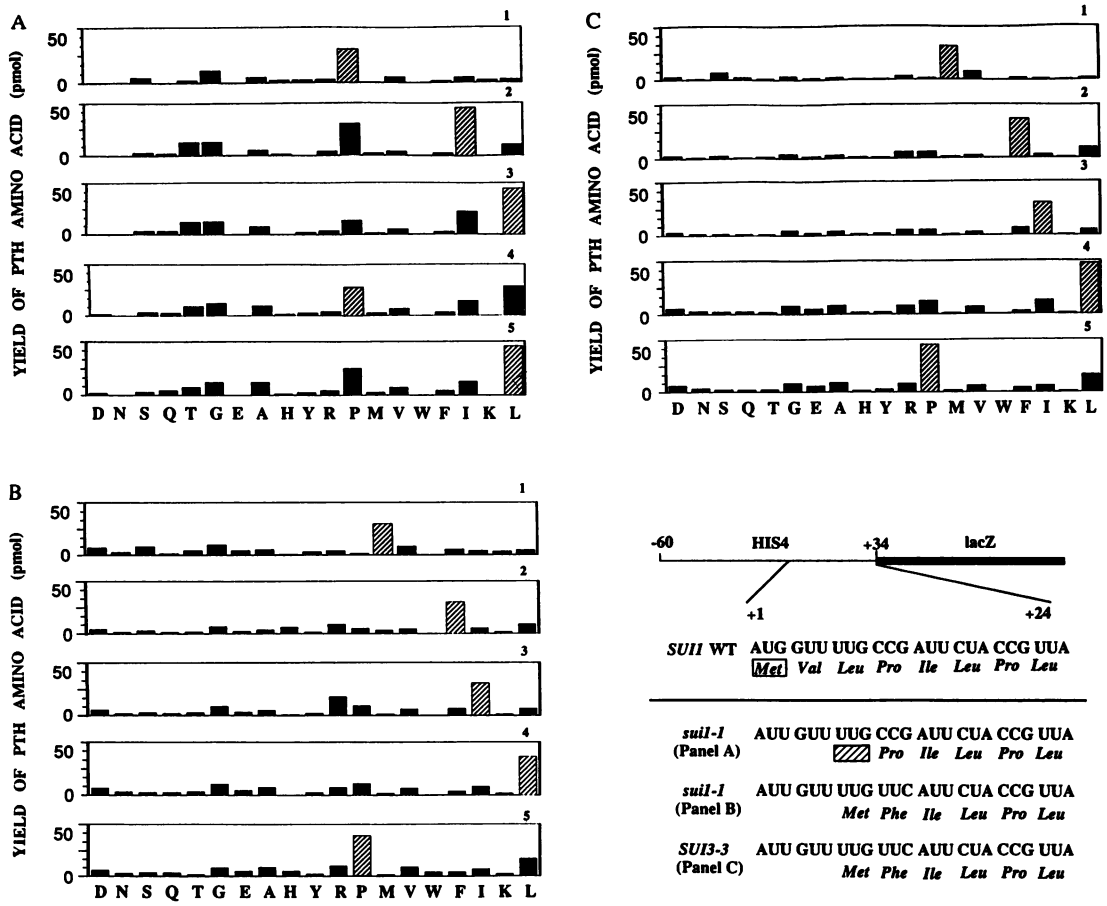

FIG. 4. Amino-terminal sequence analysis of His4–β-galactosidase protein. The His4–β-galactosidase fusion protein synthesized from initiator codon-defective *his4-lacZ* suppressor mutants was purified to homogeneity by employing the previously described method (10) or a modification of this procedure as described in Materials and Methods. Purification of His4–β-galactosidase on SDS-polyacrylamide gels resulted in a single protein band with a relative M_r of 116,000 that coelectrophoresed with either *E. coli* β-galactosidase or His4–β-galactosidase produced from an in-frame *HIS4*$^+$-*lacZ* wild-type yeast strain. Amino acid sequence analysis was determined by automated Edman degradation using an Applied Biosystems 477A liquid-phase protein-peptide sequencer with an on-line 120A phenylthiohydantoin (PTH) amino acid analyzer. The results are presented as a bar graph plotted as the total yield of each phenylthiohydantoin amino acid derivative (single-letter amino acid designations) for the first five sequencing cycles per His4–β-galactosidase protein sequenced. The results are interpreted in the lower right-hand quadrant relative to the known amino-terminal sequence Val-Leu-Pro-Ile-Leu (methionine [boxed] is cleaved posttranslationally) of the *HIS4*$^+$ wild-type protein. (A) Amino-terminal sequence (▨) of His4–β-galactosidase produced by *suil-1* suppressor strain 117-8AR4, which contains an AUU initiator codon change in the *his4-lacZ* message. (B) Amino-terminal sequence (▨) of His4–β-galactosidase produced by yeast strain HY32. Yeast strain HY32 contains the same *suil-1* suppressor allele as strain 117-8AR4 (A) but differs from 117-8AR4 by an additional mutation in the *his4-lacZ* construct [*his4*(AUU/Phe)-*lacZ*] that changes the proline codon (CCG) normally present at amino acid position +4 in the *HIS4* coding region to phenylalanine (UUC). (C) Amino-terminal sequence analysis (▨) of His4–β-galactosidase produced from yeast strain HY40. Yeast strain HY40 also contains the *his4*(AUU/Phe)-*lacZ* allele but differs from HY32 by being wild type for the *SUI1* gene and containing the *SUI3-3* suppressor allele (10).

his4-lacZ message. Obviously, methionine appears in the first cycle of the protein sequence as a result of the presence of phenylalanine in the penultimate position relative to the UUG codon at position +3. As a result of the situation of phenylalanine at this position, the methionine inserted at the UUG codon can no longer be cleaved by an aminopeptidase. The simplest interpretation of these experiments is that the effect of mutating either eIF-2β or Sui1 is to allow the standard methionine initiator tRNA to recognize a UUG codon as the site of translation initiation. Most importantly, a mutation in *suil* is qualitatively similar to a mutation in one of the known subunits of eIF-2, further demonstrating the importance of the Sui1 gene product for translation initiation, specifically, at the level of the start site selection process.

Biochemical characterization of the Sui1 gene product. As an initial biochemical characterization of the Sui1 protein, cell extracts made from *SUI1*$^+$ and *suil* suppressor strains were fractionated and probed in Western blots with antibodies made from a TrpE-Sui1 fusion protein. Figure 5A (lane 1) shows that a crude extract prepared from a wild-type strain cross-reacted with anti-TrpE-Sui1 and identified two protein species, one with an apparent M_r of less than 17,000 and a second with an apparent M_r of 26,500. Neither of these two proteins was detected when preimmune serum was substituted for our anti-TrpE-Sui1 antiserum in Western blots (data not shown). By using a crude extract that was made from a yeast strain containing the wild-type *SUI1* gene on a high-copy vector, we were able to distinguish which of these two protein species corresponded to the Sui1 protein. As

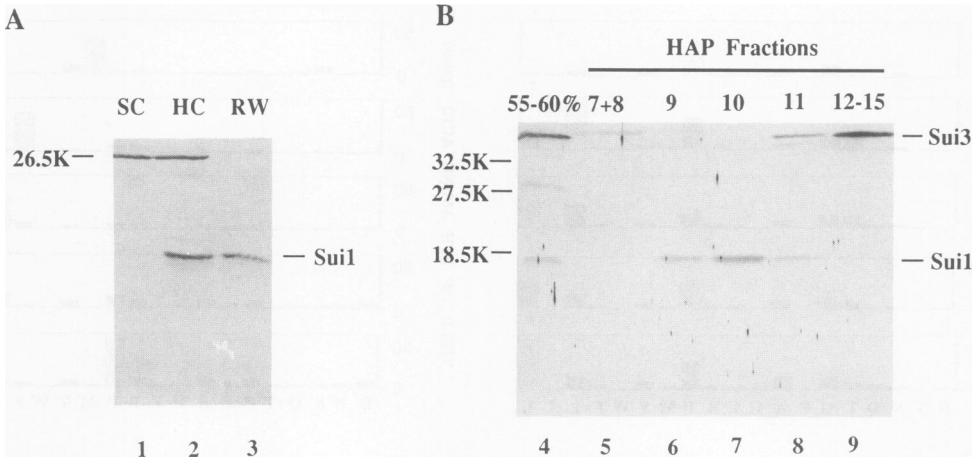

FIG. 5. Western blot analysis. Protein samples were electrophoresed on SDS-15% polyacrylamide gels with prestained molecular weight markers. The gels were transferred to nitrocellulose (0.2-μm pore size), blocked, and probed with rabbit antibodies (1:100) raised against TrpE-Sui1 protein produced in *E. coli*. Antigen-antibody complexes were visualized by using a horseradish peroxidase conjugate system. eIF-2 was partially purified from a wild-type *SUI1* strain by 55 to 60% ammonium sulfate fractionation of a 0.5 M KCl wash of ribosomes and HAP column chromatography. Similar profiles were obtained for *sui1* mutants (data not shown). (A) Lanes 1 and 2 contained 150 μg of crude extract from single-copy (SC) *SUI1* wild-type strain BC64 and multicopy (HC) *SUI1* wild-type strain BC61, which contains the 10.1-kb *Bam*HI–*Bam*HI-*Sau*3A DNA fragment (Fig. 1) in high-copy-number yeast vector YEp24. Lane 3 contained 150 μg of a 0.5 M KCl ribosomal wash (RW) fraction from *SUI1* wild-type parent strain 117-8A. (B) Lane 4 contained 150 μg of a 55 to 60% ammonium sulfate fraction of a ribosomal salt wash from *SUI1* wild-type strain 117-8A. Lanes 5 to 9 contained the HAP elution profile (100 to 600 mM KPO_4 linear gradient) from *SUI1* wild-type strain 117-8A (25 μg of protein per lane). For lanes 4 to 9, the filter was also probed with anti-Sui3 serum (1:100) and the position of the Sui3 protein (apparent M_r, 36,000; reference 10) is noted.

shown in Fig. 5A, lane 2, the polyclonal antiserum cross-reacted in a specific fashion with the apparent 17-kDa protein, as the cross-reactivity responds to an increase in *SUI1* gene dosage. This is a somewhat higher apparent M_r than expected for the Sui1 protein but nevertheless consistent with the calculated M_r (12,300) of the 108-amino-acid sequence derived from the *SUI1* gene (Fig. 2).

The crude extract prepared from the yeast strain that contains the single chromosomal copy of *SUI1* (Fig. 5A, lane 1) was further fractionated. The 26.5-kDa protein that cross-reacts with our anti-TrpE-Sui1 serum (Fig. 5A, lanes 1 and 2) remained associated with the ribosomal pellet after a salt wash of ribosomes and could be extracted from the ribosome by using methods for extracting tightly bound ribosomal proteins (data not shown). The antiserum used for Fig. 5 was directed against a TrpE-Sui1 fusion protein that fused amino acid positions +54 to +108 in the distal *SUI1* coding region in frame to the carboxyl end of the *trpE* gene. However, a different antiserum made from a smaller TrpE-Sui1 fusion protein that fused amino acid positions +71 to +108 of the distal *SUI1* coding region in frame to the carboxyl end of the *trpE* coding region weakly cross-reacted with a 26.5-kDa protein species in crude extracts or ribosomal protein extracts. This latter antiserum did cross-react with the 17-kDa protein, and the cross-reactivity with the 17-kDa protein species also responded to an increase in gene dosage (data not shown). The detection level of the 17-kDa protein species with this different antiserum was also comparable to the levels of detection shown in Fig. 5A. We assume that the differences in the patterns of cross-reactivity to the 26.5-kDa protein species observed with our two different antisera is that the antiserum directed against the larger TrpE-Sui1 fusion protein contained higher levels of antibodies against an *E. coli* protein that contaminated our gel-extracted TrpE-Sui1 protein preparation that was used for immunizations. Presumably, this protein contaminant is related in sequence to a 26.5-kDa protein from *S. cerevisiae*. In contrast, the TrpE-Sui1 fusion protein that is smaller by 17 amino acids and migrates at a slightly different position on polyacrylamide gel electrophoresis gels is less contaminated with the *E. coli* protein when extracted, and hence, the corresponding antiserum cross-reacts weakly with the 26.5-kDa protein. In addition, we attempted to determine whether the 26.5-kDa protein detected in Fig. 5A is a modified form of the 17-kDa protein. In brief, we saw no evidence that this protein is either a glycosylated or ubiquinated form of the 17-kDa protein that might account for the cross-reactivity and the M_r difference between the 17- and 26.5-kDa proteins. A search of a DNA sequence bank allowed us to detect a plant gene that is predicted to encode a gene product that is similar in length and sequence to the Sui1 gene product (see below). In light of these observations and the ability to detect increased levels of the 17-kDa protein species in response to an increased dosage of gene *SUI1*, we conclude that the 17-kDa protein is the primary gene product encoded by the *SUI1* gene.

As shown in Fig. 5A (lane 3), the 17-kDa Sui1 protein was found in a salt wash fraction of a ribosomal pellet. We estimated that greater than 60% of the Sui1 protein observed in the crude extract was found in the ribosomal salt wash fraction (Fig. 5A, compare lanes 1 and 3); the remainder of Sui1 was found in the postribosomal supernatant, and no Sui1 was detected in the pellet of salt-washed ribosomes (data not shown). For comparison, we estimated on the basis of Western blotting that approximately 10% of Sui2 protein (eIF-2α), a known subunit of yeast translation initiation factor eIF-2, was in a postribosomal supernatant with the remainder in the ribosomal salt wash fraction and that

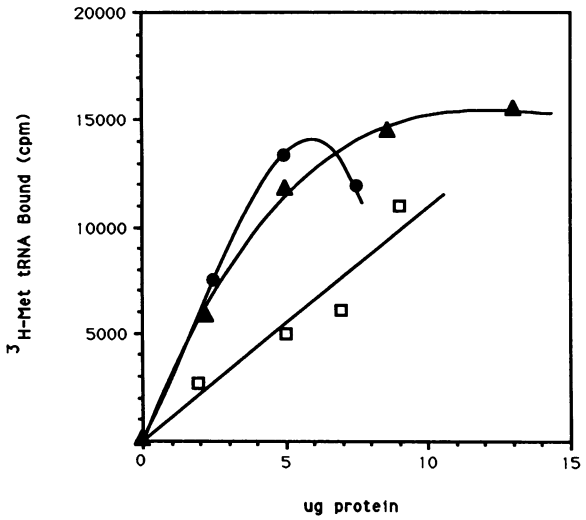

FIG. 6. In vitro assays of eIF-2 activity in partially purified *SUI1* wild-type and mutant extracts. Fractions from HAP chromatography were assayed for the ability to promote GTP-dependent binding of [^{3}H]methionyl-labeled initiator tRNA as a function of the amount of protein. Mutant extracts were subjected to the same purification scheme as shown for the wild-type extract in Fig. 5. The tRNA labeling and eIF-2 assay conditions used have been previously described (10), and the modifications used are mentioned in Materials and Methods. Protein-[^{3}H]tRNA complex formation was detected by nitrocellulose filter binding as measured by liquid scintillation counting. Identical reaction conditions lacking GTP were performed to control for nonspecific or GTP-independent binding activity at each protein concentration assayed. The number of counts per minute in the absence of GTP was subtracted from the number of counts per minute in the presence of GTP to determine GTP-dependent binding activity. Symbols: □, pooled HAP fractions 12 to 15 (Fig. 5B, lane 9) derived from wild-type strain 117-8A; ▲, HAP fraction 13 derived from isogenic *sui1-1* mutant 117-8AR4; ●, HAP fraction 13 derived from *sui1-17* mutant 138-7B. For comparison, fraction 10 from the HAP column shown in Fig. 5B (lane 7), which contained most of the Sui1 protein when assayed for eIF-2 activity (GTP-dependent binding of the initiator tRNA), had an activity of 51 cpm/μg of protein. The activities of the other HAP fractions shown in Fig. 5B are as follows: fraction 8 (lane 5), 0 cpm/μg; fraction 9 (lane 6), 20 cpm/μg; fraction 11 (lane 8), 206 cpm/μg.

```
Gos2   1 MSDLDIQIPTAFDPFAEANAGDSGAAAGSKDYVHVRIQQ 39
          ••  •    •••••   ••   • •  • • ••••
Sui1   1 MS...IENLKSFDPFAD..TGDDE.TATSN.YIHIRIQQ 32

      40 RNGRKSLTTVQGLKKEFSYNKILKDLKKEFCCNGTVVQD 78
         ••••• ••••••  •    ••• ••• • •••  • •
      33 RNGRKTLTTVQGVPEEVDLKRILKVLKKDFACNGNIVKD 71

      79 PELGQVIQLQGDQRKNVSNFLV.QAGIVKKEHIKIHGF 115
         •• • •••••••• • •  • • ••  ••••••
      72 PEMGEIIQLQGDQRAKVCEFMISQLGLQKK.NIKIHGF 108
```

FIG. 7. Comparison of the wild-type yeast *SUI1* and rice plant *GOS2* amino acid sequences (single-letter code). The 108-amino-acid sequence for the Sui1 protein is derived from the *SUI1*$^+$ DNA sequence in Fig. 2 and is compared to a 115-amino-acid sequence which was translated from the DNA sequence listed in the GenBank data base for the *GOS2* gene from the rice plant, *O. sativa* (accession no. X51910) (7a). The amino acid positions are numbered relative to the methionine (+1) encoded by the predicted AUG start codon in both genes. The two proteins were aligned by introducing gaps within the sequence to maximize homology. Identical amino acids in the putative Gos2 gene product and the Sui1 protein are designated by closed circles. The two asterisks mark amino acids that are identical between the two sequences but also highlight the two amino acid positions, +83 and +84, in the *SUI1* sequence which were found to be altered by mutation in the various *sui1* suppressor alleles (see Fig. 2 and the text for details).

approximately 50% of Sui3 (eIF-2β) was in a ribosomal salt wash fraction, with the remainder in the pellet of salt-washed ribosomes (data not shown). Thus, a ribosomal salt wash fraction is enriched with Sui1 protein, a property consistent with our finding that the Sui1 protein functions as a translation factor during the initiation process.

As previously mentioned, aside from the *sui1* suppressor gene, our genetic selection scheme also identified the *sui2* and *SUI3* suppressor genes as capable of restoring *HIS4* expression when no AUG start codon was present (4). Previous analyses showed that the *sui2* (7) and *SUI3* (10) genes encoded the α and β subunits of eIF-2, respectively. As part of these studies, we demonstrated in vitro that (i) eIF-2α and eIF-2β copurify and that these fractions contain the well-characterized biochemical activity of eIF-2, namely, GTP-dependent binding of the initiator tRNA (ternary complex formation), and (ii) *sui2* and *SUI3* suppressor mutants confer in vitro defects in ternary complex formation. In light of the genetic relatedness of the *sui1*, *sui2*, and *SUI3* suppressor mutants and identical mechanisms of suppression (Fig. 4), we further fractionated the ribosomal salt wash fraction (Fig. 5A, lane 3) to determine whether the Sui1 protein might be a subunit of eIF-2 in *S. cerevisiae* and assayed these fractions and corresponding fractions derived from *sui1* mutant strains for ternary complex formation (Fig. 6). As shown in Fig. 5B (lane 4), the Sui1 protein was observed in a 55 to 60% NH_4SO_4 fraction of the ribosomal salt wash. We have previously shown that the 55 to 60% NH_4SO_4 fraction of a ribosomal salt wash contains eIF-2 as determined by Western blotting with anti-Sui2 (7) or anti-Sui3 antibodies (Fig. 5B; reference 10). Upon further fractionation of this 55 to 60% NH_4SO_4 fraction on a HAP column, the Sui1 protein was no longer observed in the same fractions that contained most of the Sui3 (eIF-2β) protein. Sui1 protein eluted slightly earlier from HAP (Fig. 5B, lanes 6 to 8) than did Sui3 (Fig. 5B, lanes 8 and 9). In addition, the fractions that contained Sui3 protein also contained Sui2 protein whereas no Sui2 protein was detected in the fractions that contained the Sui1 protein as determined by Western blotting of these fractions with anti-Sui2 serum (data not shown). The HAP fraction containing most of the wild-type Sui1 protein (Fig. 5B, lane 7) possessed little or no eIF-2 activity (see the legend to Fig. 6 for details). The eIF-2 activity (Fig. 6) was located in the latter fractions, where the Sui2 (data not shown) and Sui3 proteins were most abundant (Fig. 5B, lane 9). Assays of eIF-2 activity in similar HAP fractions derived from extracts of *sui1* mutants did not result in reduced GTP-dependent binding activity for the initiator tRNA (Fig. 6). Although the fractionation profiles we observed are properties consistent with the idea that the *SUI1* gene encodes a protein that is involved in the translation process, the Sui1 protein does not appear to be a subunit of eIF-2 that is required for ternary complex formation, as Sui1 did not appear in HAP fractions which contain eIF-2 and mutations in Sui1 did not alter eIF-2 ternary complex formation activity. This is consistent with the established subunit requirement of eIF-2 for ternary complex formation (GTP-dependent binding of initiator tRNA; 24). eIF-2 is composed of three subunits, α, β, and γ, of which the γ subunit is believed to be an approximately 49-kDa protein in

both *S. cerevisiae* (1) and mammals (24). Therefore, although the *sui2* and *SUI3* suppressor loci identified the α and β subunits of eIF-2 and arose from the same genetic selection as *sui1*, the *sui1* suppressor gene does not encode the γ subunit of eIF-2. In light of other studies we have presented, we suggest that the *SUI1* gene product encodes an additional translation initiation factor that normally functions in concert with eIF-2 and the initiator tRNA to arrive at ribosomal recognition of the start codon.

The Sui1 protein is homologous to an amino acid sequence derived from the *GOS2* DNA sequence. Searches of protein sequence data bases identified no previously characterized gene product related to the *SUI1* gene product. However, a search of the GenBank DNA data base with the *SUI1*$^+$ DNA sequence indicated that the *SUI1* DNA sequence was related to the *GOS2* gene from the rice plant, *Oryza sativa* (accession no. X51910) (7a). Translation of the DNA sequence of the *GOS2* gene and comparison of this 115-amino-acid open reading frame to the Sui1 amino acid sequence indicated that Sui1 is 61% identical to the putative *GOS2* gene product (Fig. 7). Interestingly, the mutations identified in *sui1* suppressor alleles (Fig. 2) alter amino acids that are identical in the same relative position in the putative *GOS2* protein sequence (Fig. 7). The observation that *GOS2* is similar to *SUI1* in coding length and amino acid sequence further corroborates our assignment of the 108-amino-acid sequence and the 17-kDa protein observed in Western blots as the primary Sui1 gene product, as a similar-size gene product must be present in rice and encoded by *GOS2*. Unfortunately, aside from the fact that it is a constitutively expressed gene, the function of *GOS2* in rice is not known. However, given the significant identity and the similarity in the lengths of these two amino acid sequences, we anticipate that the *GOS2* gene product is the plant homolog of the yeast Sui1 protein and has a similar function during the translation initiation process in plants.

DISCUSSION

By using a genetic reversion scheme at the *HIS4* locus in *S. cerevisiae*, we attempted to identify by mutation components of the translation initiation complex that are important for ribosomal recognition of an initiator codon. The basic strategy was that mutant components would confer altered specificities on the translation initiation complex. Thus, the ribosome would no longer bypass a mutant *his4* initiator region but would recognize and initiate at a non-AUG codon, restoring a His$^+$ phenotype. Genetic analysis of 67 extragenic suppressor mutants isolated from haploid yeast strains identified only three unlinked suppressor loci, *sui1*, *sui2*, and *SUI3*, each of which, when mutated, was capable of restoring *HIS4* expression in the absence of an AUG start codon (4). As part of this analysis, other genetic studies suggested that the gene products encoded by the *sui1*, *sui2*, and *SUI3* genes are specific in their suppression mechanism and share a related function. (i) Each suppressor mutant was incapable of suppressing known frameshift, missense, and nonsense mutations but was capable of suppressing all possible point mutations of the AUG start codon at *his4*. (ii) Each suppressor was incapable of suppressing a *his4* allele that contained a deletion from positions −4 to +9, suggesting that the site of suppression immediately surrounds the mutant initiator region. (iii) All pairwise combinations of *sui1*, *sui2*, and *SUI3* suppressor genes in haploid cells conferred lethality, indicating that all three gene products either functionally interact or function in a related pathway. These data, in light of the limited and genetically saturated spectrum of suppressor genes obtained by this genetic reversion scheme, suggested that this approach was highly specific in identifying gene products that function at a related step in the translation initiation pathway.

The molecular and biochemical characterization of the *sui2* and *SUI3* genes and gene products validated the initial assumptions of our genetic selection scheme and pointed to the related and specific functions of these gene products during the translation initiation process. The fact that the *sui2* (7) and *SUI3* (10) genes encode the α and β subunits of eIF-2, respectively, demonstrated that aside from initiator tRNA-binding activity (13), eIF-2 has an additional function during the translation initiation process, i.e., ribosomal recognition of a start codon. This is consistent with the known in vitro properties of eIF-2, which binds the initiator tRNA and remains closely associated with the scanning ribosome up until the time of 80S complex formation. Furthermore, suppressor mutations in *SUI3* were shown to map to a putative zinc finger motif, suggesting that a nucleic-acid-binding domain as part of eIF-2 was involved in stabilizing the codon-anticodon interaction between the AUG and the initiator tRNA during the recognition process. The net effect of suppressor mutations in *SUI3* would then be to increase the retention time of the ribosome at the UUG codon in the early *HIS4* coding region by stabilizing a mismatched codon-anticodon interaction between UUG and the initiator tRNA. Suppressor mutations in *sui2* might also afford such direct effects or, alternatively, alter β function through a protein-protein interaction to arrive at non-AUG initiation events.

It therefore seemed logical to expect that the third and last suppressor gene to be characterized from our genetic reversion analysis of haploid yeast strains, *sui1*, might encode an additional protein required for ribosomal recognition of a start codon, perhaps the γ subunit, the third and last subunit of eIF-2, as defined by being required in addition to α and β to promote ternary complex formation (GTP-dependent binding of the initiator tRNA). However, the data presented in this report are inconsistent with the notion that the *sui1* gene encodes the γ subunit of eIF-2. (i) The Sui1 protein is too small to be the γ subunit of eIF-2. The Sui1 protein has an apparent M_r of less than 17,000 (Fig. 5), compared with the apparent M_r of eIF-2γ, which is reported to be in excess of 49,000 in both *S. cerevisiae* (1) and mammalian (24) cells. (ii) The Sui1 protein did not copurify with eIF-2 fractions that maintain GTP-dependent binding activity for the initiator tRNA (Fig. 5). (iii) eIF-2, when partially purified from *sui1* mutants, still maintained wild-type levels of ternary complex formation activity (Fig. 6), in contrast to eIF-2 derived from *sui2* (7) or *SUI3* (10) mutants, which show either a decrease in or complete loss of this activity in vitro.

This leads to the suggestion that the *sui1* gene encodes an additional factor that acts in concert with eIF-2 at a subsequent step in the translation initiation process, namely, ribosomal recognition of a start codon. Our data are consistent with this notion. Conditional *sui1* suppressor mutants exhibit altered polysome profiles, as expected of a gene product that functions during the early steps of translation initiation (Fig. 3B). In fact, this profile parallels the polysome profile of a conditional *sui2* mutant which encodes a thermolabile eIF-2α gene product known to function during the initiation step (Fig. 3C). Control experiments (data not shown) demonstrated that under the experimental conditions described, a Ts$^-$ mutant unrelated to translation had identical and normal polysome profiles at both the permissive and

restrictive temperatures. This indicates that the alteration in polysome profiles observed with *sui1* and *sui2* mutants at the restrictive temperature is specific for the functions of the encoded gene products and is not an indirect effect of a Ts^- mutation. Another part of our analysis that supports our suggestion that the *sui1* gene encodes a factor that functions during start codon recognition stems from amino-terminal sequence analysis of the His4–β-galactosidase protein. As observed for *sui2* (7) and *SUI3* (10) mutants, *sui1* suppressor strains result in initiation at a UUG codon in the early *his4* coding region (Fig. 4A). Most importantly, by changing the penultimate amino acid next to this UUG codon from a Pro to a Phe codon, we were able to demonstrate that the amino acid corresponds to Met, which indicates that the initiator tRNA is used to initiate at the UUG codon. This observation is important for a number of reasons. (i) It shows that the effect of mutating *sui1* does not result in an aberrant mechanism of translation initiation at *his4* but rather the normal translation initiation machinery is used. In light of other data presented, this further suggests that the Sui1 protein functions during the mechanism of translation initiation in *S. cerevisiae*. (ii) The observation that *SUI3* suppression events also utilize the initiator tRNA to initiate at UUG (Fig. 4C) shows that the mechanism of suppression is common to *sui1* and *SUI3* suppression, and therefore, the Sui1 protein appears to have a function related to recognition of a start codon by eIF-2. This is in complete agreement with our genetic studies of *sui1*, *sui2*, and *SUI3*, as mentioned above, which suggested that the suppressors employ a similar mechanism of suppression and functionally interact during a related steps of the translation initiation process. (iii) This amino-terminal sequence analysis makes an important mechanistic distinction for the role of eIF-2 during the initiation process. The observation that a mutation in eIF-2 affords a mismatched anticodon-codon interaction between the initiator tRNA and UUG, as opposed to allowing a Leu tRNA to initiate at UUG, represents more definitive evidence that eIF-2 functions during start site selection. Clearly, eIF-2 activity is not limited to tRNA binding.

One simple interpretation of our analysis is that Sui1 functions with eIF-2 and the initiator tRNA to establish initiation at an AUG codon. Although our data appear to eliminate the possibility that the Sui1 protein is a subunit of eIF-2 that is required for ternary complex formation, they do not rule out the possibility that it is part of a mutimeric complex that includes eIF-2 and the initiator tRNA that dictates the recognition process. Our genetic observations may, in fact, suggest this; specifically, the data that show that *sui1 sui2* and *sui1 SUI3* double mutants confer lethality on haploid yeast strains (4). The cofractionation and chromatography of Sui1 relative to eIF-2 (Fig. 5) may indicate a weak interaction that is easily dissociated on a HAP column. Our attempts to show a specific interaction by coimmunoprecipitation of Sui1 protein with either anti-eIF-2α or anti-eIF-2β serum or coimmunoprecipitation of eIF-2α or eIF-2β protein with anti-Sui1 serum have not been successful (unpublished data). However, these experiments may have limitations, as our antiserum directed against eIF-2α does not coimmunoprecipitate eIF-2β although anti-eIF-2β does precipitate eIF-2α. More extensive biochemical studies are needed to define the function of Sui1 and its relationship with other translation initiation components.

Sequence searches indicate that the *sui1* gene is not one of the approximately 35 ribosomal protein genes that have been cloned from *S. cerevisiae*, nor does Sui1 appear to be related to other known ribosomal proteins identified in other organisms. We have also examined the *sui1* gene for a number of characteristics that are frequently found associated with ribosomal genes (32). The *sui1* gene is not duplicated, nor does it contain an intron. It is a small protein, as are many ribosomal proteins, and has a somewhat basic amino acid composition (15% Arg or Lys). The *sui1* gene does appear to possess an Abf1-like upstream activation site sequence in its promoter; this sequence has been found only in the promoter regions of approximately four yeast ribosomal protein genes (32). There appear to be a number of sequences in the *sui1* 5′ noncoding region that could be candidates for the Rap1 DNA-binding site which is more frequently observed among promoter regions of ribosomal protein genes (32). Although it would be most interesting if Sui1 were a ribosomal protein, as no structural component of the eukaryotic ribosome has ever been directly implicated in translation initiation, our initial biochemical analysis of the Sui1 gene product appeared to rule out this possibility, as Sui1 was completely dissociated from the ribosome by a salt wash (Fig. 5A).

This leads us to believe that *sui1* encodes a translation initiation factor. Given the apparent M_r of the Sui1 protein, calculated as part of this analysis, we compared this to the apparent M_rs listed for the approximately 25 proteins believed to compose the various mammalian translation initiation factors (24). Three proteins possess M_rs consistent with that of Sui1: eIF-1, 15,000; eIF-1A, 17,600; eIF-5A, 16,700. Of these, we know that Sui1 is not eIF-5A, as two duplicated genes that encode yeast eIF-5A, *TIF51A* and *TIF51B* (26), have been isolated and neither gene is the *sui1* locus. In addition, we probed mammalian eIF-1 (kindly provided by J. W. B. Hershey) with anti-Sui1 and found no cross-reactivity, although the absence of cross-reactivity might be expected, given the evolutionary distance between *S. cerevisiae* and mammals. Therefore, the Sui1 protein could be the yeast equivalent of eIF-1 or eIF-1A, which may make sense, as both factors have been implicated in stimulation of the 40S preinitiation complex (27, 31). If this were the case, the observation would prove to be quite interesting, as little is known about either of these two factors. Neither has been considered to be required for in vitro translation initiation, whereas Sui1 clearly performs a required role in vivo. Alternatively, Sui1 may represent a translation initiation factor that has not been identified by biochemical studies. In any event, our analysis strongly implicates a function for the Sui1 gene product during ribosomal recognition of a start codon. These studies further point to the effectiveness of a genetic system for identification of components of the translation initiation complex that act at a specific step during the scanning process in *S. cerevisiae*.

ACKNOWLEDGMENTS

We thank Ed Pabich for technical assistance, Mark Cigan and Alan Hinnebusch for help with the eIF-2 assays, Tom Petes for the rDNA probes, Keith Gulyas and Mark Goebl for performing computer searches, and Tom Blumenthal for helpful comments on the manuscript.

This work was supported by Public Health Service grant GM32263 from the National Institutes of Health to T.F.D.

REFERENCES

1. **Ahmad, M. F., N. Nasrin, M. K. Bagchi, I. Chakravarty, and N. K. Gupta.** 1985. A comparative study of the characteristics of eIF-2 and eIF-2-ancillary factor activities from yeast *Saccharomyces cerevisiae* and rabbit reticulocytes. J. Biol. Chem. **260:** 6960–6965.
2. **Baim, S. B., D. F. Pietras, D. C. Eustice, and F. Sherman.** 1985. A mutation allowing an mRNA secondary structure diminishes

translation of *Saccharomyces cerevisiae* iso-1-cytochrome *c*. Mol. Cell. Biol. **5**:1839–1846.
3. **Burgin, A. B., K. Parodos, D. J. Lane, and N. R. Pace.** 1990. The excision of intervening sequences from Salmonella 23S ribosomal RNA. Cell **60**:405–414.
4. **Castilho-Valavicius, B., H. Yoon, and T. F. Donahue.** 1990. Genetic characterization of the *Saccharomyces cerevisiae* translational initiation suppressors *sui1*, *sui2* and *SUI3* and their effects on *HIS4* expression. Genetics **124**:483–495.
5. **Cigan, A. M., L. Feng, and T. F. Donahue.** 1988. $tRNA_i^{Met}$ functions in directing the scanning ribosome to the site of translation. Science **242**:93–97.
6. **Cigan, A. M., E. K. Pabich, and T. F. Donahue.** 1988. Mutational analysis of the *HIS4* translational initiator region in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:2964–2975.
7. **Cigan, A. M., E. K. Pabich, L. Feng, and T. F. Donahue.** 1989. Yeast translation initiation suppressor *sui2* encodes the α subunit of eukaryotic initiation factor 2 and shares sequence identity with the human α subunit. Proc. Natl. Acad. Sci. USA **86**:2784–2788.

7a. **de Pater, B. S., and R. Schilperoort, and L. A. M. Hensgens.** Unpublished data.

8. **Dieckmann, C. L., and A. Tzagoloff.** 1985. Assembly of the mitochondrial membrane system. J. Biol. Chem. **260**:1513–1520.
9. **Donahue, T. F., and A. M. Cigan.** 1988. Genetic selection for mutations that reduce or abolish ribosomal recognition of the *HIS4* translational initiator region. Mol. Cell. Biol. **8**:2955–2963.
10. **Donahue, T. F., A. M. Cigan, E. K. Pabich, and B. Castilho-Valavicius.** 1988. Mutations at a Zn(II) finger motif in the yeast eIF-2β gene alter ribosomal start-site selection during the scanning process. Cell **54**:621–632.
11. **Donahue, T. F., P. J. Farabaugh, and G. R. Fink.** 1983. The nucleotide sequence of the *HIS4* region of yeast. Gene **18**:47–59.
12. **Ernst, H., R. F. Duncan, and J. W. B. Hershey.** 1987. Cloning and sequencing of complementary DNAs encoding the α-subunit of translation initiation factor eIF-2. J. Biol. Chem. **262**: 1206–1212.
13. **Gupta, N. K., C. L. Woodley, Y. N. Chen, and K. K. Bose.** 1973. Protein synthesis in rabbit reticulocytes: assays, purification, and properties of different ribosomal factors and their roles in polypeptide chain initiation. J. Biol. Chem. **248**:4500–4511.
14. **Hartwell, L. H., and C. S. McLaughlin.** 1969. A mutant of yeast apparently defective in the initiation of protein synthesis. Proc. Natl. Acad. Sci. USA **62**:468–474.
15. **Hershey, J. W. B.** 1991. Translational control in mammalian cells. Annu. Rev. Biochem. **60**:717–754.
16. **Hewick, R. M., M. W. Hunkapiller, L. E. Hood, and W. J. Dreyer.** 1981. A gas-liquid solid phase peptide and protein sequenator. J. Biol. Chem. **256**:7790–7997.
17. **Kozak, M.** 1978. How do eucaryotic ribosomes select initiation regions in messenger RNA? Cell **15**:1109–1123.
18. **Kozak, M.** 1980. Evaluation of the 'scanning model' of initiation of protein synthesis in eucaryotes. Cell **22**:7–8.
19. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labelled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
20. **Orr-Weaver, T. L., J. K. Szostak, and R. J. Rothstein.** 1983. Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101**:228–245.
21. **Pathak, V. K., P. Nielsen, H. Trachsel, and J. W. B. Hershey.** 1988. Structure of the β subunit of translation initiation factor eIF-2. Cell **54**:633–639.
22. **Petes, T. D., L. M. Hereford, and K. G. Skryabin.** 1978. Characterization of two types of yeast ribosomal DNA genes. J. Bacteriol. **134**:295–305.
23. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
24. **Safer, B.** 1989. Nomenclature of initiation, elongation and termination factors for translation in eukaryotes. Eur. J. Biochem. **186**:1–3.
25. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
26. **Schnier, J., H. G. Schwelberger, Z. Smit-McBride, H. A. Kang, and J. W. B. Hershey.** 1991. Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:3105–3114.
27. **Schreier, M. H., B. Erni, and T. Staehelin.** 1977. Initiation of mammalian protein synthesis. I. Purification and characterization of seven initiation factors. J. Mol. Biol. **116**:727–753.
28. **Sherman, F.** 1990. Protein modifications and mitochondrial import of yeast cytochrome c: an overview, p. 637–646. *In* J. E. G. McCarthy and M. F. Tuite (ed.), Post-transcriptional control of gene expression. Springer-Verlag KG, Berlin.
29. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1972. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Sherman, F., and J. W. Stewart.** 1982. Mutations altering initiation of translation of yeast iso-1-cytochrome c: contrasts between the eukaryotic and prokaryotic initiation process, p. 301–333. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast Saccharomyces: metabolism and gene expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Thomas, A., H. Goumans, H. O. Voorma, and R. Benne.** 1980. The mechanism of action of eukaryotic initiation factor 4C in protein synthesis. Eur. J. Biochem. **107**:39–45.
32. **Woolford, J. L., Jr., and J. R. Warner.** 1982. The ribosome and its synthesis. *In* J. R. Broach, E. W. Jones, and J. Pringle (ed.), The molecular and cellular biology of the yeast Saccharomyces: genome dynamics, protein synthesis, and energetics, in press. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.