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Genetics

Yeast translation initiation suppressor *sui2* encodes the α subunit of eukaryotic initiation factor 2 and shares sequence identity with the human α subunit

(scanning model/start site selection/phosphorylation/initiation factors)

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ABSTRACT **Genetic reversion of *HIS4* initiator codon mutations in yeast has identified three unlinked genes, *sui1*, *sui2*, and *SUI3* (*su*ppressors of *i*nitiator codon mutations), which when mutated confer the ability to initiate translation at *HIS4* despite the absence of an AUG start codon. We have previously demonstrated that the *SUI3* gene encodes the β subunit of the eukaryotic initiation factor 2 (eIF-2) and that mutations at a Zn(II) finger motif of *SUI3* alter the start site selection process in yeast. In this report, molecular and biochemical characterizations show that the *sui2* suppressor gene encodes the α subunit of eIF-2. The amino acid sequence of *sui2* is 58% homologous to that encoded by the cDNA of the human eIF-2α. Mutations in the *sui2* suppressor alleles occur in the amino-terminal portion of the protein and change amino acids that are identical at the same relative position in the yeast and human proteins. Protein sequence analysis shows that a *sui2* mutant yeast strain allows initiation at a UUG codon in the absence of an AUG codon at *HIS4*. These data further suggest that eIF-2 is an important component of the preinitiation complex that mediates ribosomal recognition of a start codon during the scanning process.**

The eukaryotic translation initiation factor 2 (eIF-2) is composed of three nonidentical subunits: α, β, and γ (1). One role of eIF-2 established by biochemical studies (2) is that it functions during the early steps of translation initiation by forming a ternary complex with GTP and initiator tRNA. This complex then binds the small 40S ribosomal subunit, which in turn binds the 5′ end of eukaryotic mRNA. According to the scanning model (3, 4), this preinitiation complex then scans the leader region until the first AUG codon is reached whereupon translation begins.

Recent genetic studies in our laboratory have provided biological evidence that eIF-2 may also function in ribosomal selection of the initiator codon during the scanning process (5). By reverting his4$^-$, his4$^-$-lacZ *Saccharomyces cerevisiae* initiator codon mutants (His$^-$, white), three unlinked genes, *sui1*, *sui2*, and *SUI3*, were identified that when mutated act in trans to restore both *his4* and *his4-lacZ* expression [His$^+$, blue revertant colonies on synthetic dextrose minus histidine and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) indicator plates, respectively] despite the absence of an AUG start codon in each message. Molecular and biochemical characterizations showed that the *SUI3* suppressor gene encodes the β subunit of eIF-2 (5), being 42% homologous to the human β subunit of eIF-2 (6) and mutations that confer suppression map to a zinc(II) finger motif in the carboxyl end of the encoded β subunit. Further analysis demonstrated that these mutations in *SUI3* confer the ability to the ribosome to no longer bypass the mutant *his4* initiator region but now recognize and initiate at UUG in the early *his4* coding region (5).

In this report, we present the characterization of the *sui2* suppressor gene. Our study shows that *sui2* encodes the α subunit of eIF-2 and mutations in α also confer an alteration in start site selection by allowing initiation at a UUG codon in the mutant *his4* message. As observed for the yeast and human β subunit of eIF-2, the yeast α subunit shows considerable identity to the amino acid sequence derived from a cDNA encoding the human α subunit of eIF-2 (7) and mutations in *sui2* suppressor genes that restore *his4* expression change amino acids that are identical at the same position in the human eIF-2α sequence. In light of similarities between the yeast and mammalian initiation processes (3, 4, 8, 9), these studies further demonstrate the functional significance of eIF-2 in mediating the general mechanism of start-site selection in all eukaryotic organisms.

MATERIALS AND METHODS

Yeast Strains. The wild-type yeast strains 117-8A [*MATα his4-303* (AUU) *ura3-52* (*URA3*$^+$; AUU *his4-lacZ*)] and 123-4A [*MATα his4-301*(ACG) *ura3-52* (*URA3*$^+$; ACG *his4-lacZ*)] and the isogenic spontaneous *sui2* revertant strains 117-8AR20 (*sui2-1*) and 123-4AR31 (*sui2-2*) were used as starting strains for this analysis. All strains are related to TD28 (*MATa ura3-52 inol-13*), an ascospore derivative of S288C (*MATα*). The genetic methods used to identify and characterize the *sui2* revertant strains have been reported (5). Aside from the suppressor phenotype associated with the *sui2* revertants, these strains are temperature-sensitive (ts) for growth on enriched medium (yeast extract/peptone/dextrose) at 37°C and this phenotype cosegregates with the suppressor phenotype in genetic crosses.

Recombinant DNA Methods. A YEp13 clone bank (10) composed of wild-type yeast genomic DNA was used to isolate the *SUI2*$^+$ wild-type gene by transformation (11) of a *leu2*$^-$ ascospore derivative of 117-8AR20 (*sui2-1*) and screening for complementation of the ts phenotype. Subcloning and localization of the *SUI2*$^+$ gene was performed with the single copy *URA3*$^+$ yeast vector YCp50. The *sui2-1* and *sui2-2* mutant alleles were isolated from *ura3-52* ascospore derivatives of 117-8AR20 and 123-4AR31, respectively, by the integration/excision method (12) using the distal *Bgl* II/*Bam*HI DNA fragment derived from the *SUI2*$^+$ gene (see Fig. 1) in the integrating *URA3*$^+$ yeast vector YIp5.

The relationship of the cloned DNA fragment to the *SUI2* locus was established by genetic analysis. The wild-type 2.7-kilobase (kb) *Bam*HI DNA fragment (see Fig. 1) was ligated into the *Bam*HI YIp5 and used to transform (13) yeast

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Abbreviations: eIF-2α, α subunit of eukaryotic initiator factor 2; ts, temperature sensitive; PTH, phenylthiohydantoin.

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strain TD28. Ura$^+$ transformants were crossed to a *ura3-52, sui2-1* suppressor strain and analyzed by tetrad analysis. All Ura$^+$ transformants were temperature resistant as opposed to Ura$^-$ meiotic products, which were ts, indicative of the Ura$^+$ phenotype being linked to the *SUI2* locus.

The proximal *Bam*HI/*Bgl* II *SUI2*$^+$ DNA fragment (see Fig. 1) was ligated into the *Bam*HI site of plasmid p546. Plasmid p546 consists of the *Escherichia coli lacZ* coding region contained on a *Bam*HI/*Sal* I DNA restriction fragment from pMC1856 (14) ligated into the *Bam*HI/*Sal* I sites of YCp50. As a result of this construction the *SUI2* coding region is fused in-frame with the *lacZ* coding region. This plasmid was used to transform TD28 and transformants were analyzed for β-galactosidase activity as described (9).

Plasmid pSUI2 contains the *SUI2*$^+$ gene on a 2.7-kb *Bam*HI DNA fragment in the *Bam*HI site of pBR322 and was used for gene disruption experiments. The 1.1-kb DNA restriction fragment containing the *URA3*$^+$ (15) gene was adapted with *Bam*HI ends and was ligated into the *Bgl* II site within the *SUI2* coding region contained on pSUI2 to construct pSUI2::URA3. This allows the *SUI2::URA3*$^+$ disrupted gene to be isolated as a 3.8-kb *Bam*HI DNA fragment (Fig. 2), which was used to transform (13) the diploid strain EKP1 (*MATα/MATa HIS4*$^+$*/his4-401 ura3-52/ura3-52 LEU2*$^+$*/leu2-3,-112 INO1*$^+$*/inol-13*) to *URA3*$^+$ to generate strain EKP53, which is isogenic to EKP1 with the exception of a disrupted copy of *SUI2* (*SUI2::URA3*$^+$) on one of the chromosomal homologues (see Fig. 2).

DNA sequence and Southern analysis were performed as described (9).

Biochemical and Immunological Methods. Antibodies directed against the *SUI2* gene product were generated from TrpE-Sui2 fusion products expressed in *E. coli*. The distal *SUI2*$^+$ coding region from amino acid positions +76 to +304 contained on the 1.9-kb *Sal* I/*Bam*HI DNA fragment (see Fig. 1) was ligated in-frame to the carboxyl end of the *E. coli trpE* coding region as part of the pATH3 expression vector (16). The methods used for extracting the antigen and immunizations have been described (5). The specificity and the titer of the antiserum were determined by Western blot analysis (17) using extracts prepared from the single copy *SUI2*$^+$ strain BC-36 (*MATα, his4-401, ura3-52, leu-2-3,-112*) and the isogenic strain BC-52, which contains the *SUI2*$^+$ gene on the high copy *LEU2*$^+$ yeast vector YEp13.

To detect phosphorylation of eIF-2α, yeast strain 117-8A was labeled with [^{32}P]orthophosphate (18) and cell extracts were prepared as described (5) with the exception that phosphatase inhibitors [50 mM β-glycerol phosphate (pH 7.2) and 100 μM sodium orthovanadate] were added. Sui2 was immunoprecipitated from crude extracts (500 μg of total protein) in a 250-μl reaction mixture using anti-TrpE-Sui2 antibodies (diluted 1:50) in PBS (20 mM NaH_2PO_4, pH 7.2/0.15 M NaCl) on ice for 60 min. Fifty microliters of *Staphylococcus* A cells (10% suspension) was added and incubated on ice for 30 min and pellets were washed three times with 500 μl of PBS containing 0.5 M NaCl, 0.1% SDS, 1% Triton X-100. Pellets were electrophoresed on 10% SDS/polyacrylamide gel containing 6 M urea (19). Gels were blotted to nylon filters and probed with anti-TrpE-Sui2 (diluted 1:150) and filters were exposed to x-ray film with an intensifying screen at −70°C for 1–2 days.

His4 β-galactosidase was purified to homogeneity from yeast strain 117-8AR20 (*sui2-1*) and the amino-terminal sequence was determined as described (5). β-Galactosidase-specific activities of crude extracts were approximately 4 units for the parent strain 117-8A, 25 units for the *sui2-1* suppressor strains 117-8AR20, and 450 units for *HIS4-lacZ SUI2* wild-type strains.

RESULTS

Characterization of the Wild-Type *SUI2* Gene. The *SUI2* wild-type gene was identified as a 5.7-kb DNA restriction fragment (Fig. 1) from a YEp13 genomic clone bank based on its ability to complement the recessive ts phenotype and poor growth characteristics at 23°C associated with *sui2* mutant strains. The ability to complement the *sui2* ts phenotype was localized to a 2.5-kb *Eco*RI/*Bam*HI subclone and deletion analysis suggested that *SUI2* was contained within the *Eco*RI/*Bgl* II restriction sites (Fig. 1). Genetic analysis of wild-type yeast strains transformed with the 2.7-kb *Bam*HI DNA fragment as part of the integrating *URA3*$^+$ YIp5 vector demonstrated that this fragment was derived from the genomic *sui2* region. Southern blot analysis showed that this region is unique in the haploid yeast genome (Fig. 2).

The DNA sequence of this region is presented in Fig. 3. An open reading frame that begins with an ATG is identified that would encode a 304-amino acid protein with a calculated M_r of 34,700. An in-frame fusion of the proximal *Bam*HI/*Bgl* II DNA fragment to the *lacZ* coding region promotes the synthesis of β-galactosidase in yeast (≈135 units). Gene disruption (22) of one copy of this open reading frame in a wild-type diploid strain (Fig. 2, lane EKP53) results in two viable and two inviable meiotic products upon sporulation. Therefore, this 304-amino acid coding region is transcribed and translated in yeast and encodes a unique essential gene product.

Characterization of the Mutant *sui2* Genes. The identical DNA region was isolated from *sui2* suppressor strains. DNA sequence analysis of the complete coding region from each fragment identifies a single base change that alters the amino acid sequence. The sequence from the *sui2-1* strain contains a proline (CCA) to serine (TCA) change at amino acid position +14 and the sequence from the *sui2-2* strain contains a valine (GTC) to phenylalanine (TTC) change at position +20 (Fig. 3). Therefore, the 304-amino acid open reading frame encodes the *sui2* gene product.

Amino Acid Comparison of Sui2 to Human eIF-2α. The 304-amino acid open reading frame derived from the *SUI2*$^+$

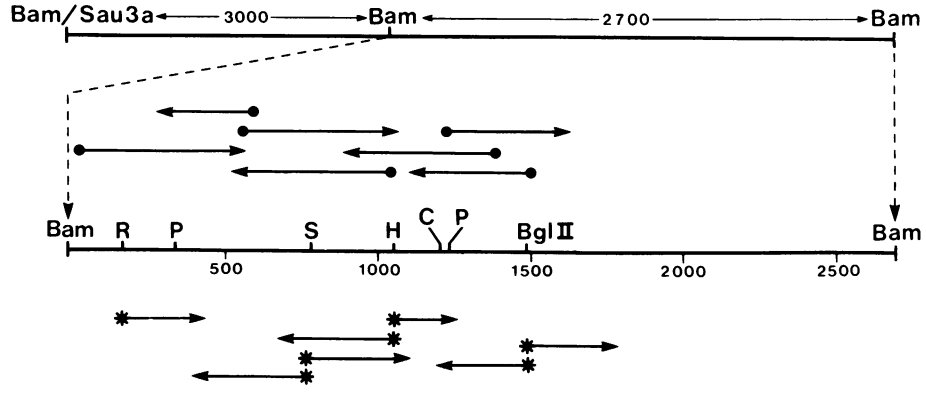

FIG. 1. Restriction map and sequencing strategies for the *SUI2* wild-type clone. The 5.7-kb DNA was identified from a YEp13 clone bank based on the ability to complement the ts phenotype associated with a *sui2* suppressor strain. The *SUI2* gene was localized to the 2700-base-pair *Bam*HI DNA fragment and was used for additional characterization. The direction and extent of DNA sequencing by the dideoxynucleotide (20) and Maxam and Gilbert methods (21) are indicated as arrows with solid circles and asterisks, respectively. Bam, *Bam*HI; R, *Eco*RI; P, *Pvu* II; S, *Sal* I; H, *Hin*dIII; C, *Cla* I.

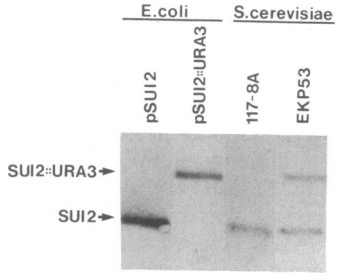

FIG. 2. Southern analysis. Genomic DNA (10 μg) isolated from yeast strains 117-8A and EKP53 and the plasmid DNA (20 ng) from pSUI2 and pSUI2::URA3 grown in *E. coli* were digested with *Bam*HI, coelectrophoresed on a 0.6% agarose gel, transferred to nitrocellulose, and probed with nick-translated *SUI2*$^+$ DNA (2.7-kb *Bam*HI DNA fragment). The high molecular weight band corresponds in size to the 3.8-kb *Bam*HI DNA fragment that contains the disrupted *SUI2* gene (*SUI2*::*URA3*) and the lower molecular weight band corresponds to the wild-type gene (*SUI2*) present on the 2.7-kb *Bam*HI DNA fragment. The diploid EKP53 contains both a wild-type and a disrupted copy of the *SUI2* gene.

wild-type clone is similar in sequence to a previously reported 315-amino acid open reading frame present in a cDNA that encodes the α subunit of the human eIF-2 (7). In addition, mutations identified in the *sui2-1* and *sui2-2* alleles alter amino acids that are identical at the same position in the encoded human α sequence (Figs. 3 and 4). Overall, 58% of the yeast and human amino acid positions are identical (Fig. 4) [79% when conservative substitutions (23) are considered]. Significant sequence conservation is observed at the amino-terminal third of the protein (92% identical and conservative amino acids). Within this region, the yeast and human amino acid sequences are identical between positions +41 and +59, which contains the Ser-52 amino acid, a site that has been proposed to be phosphorylated (24, 25) in higher eukaryotes. (Generally referred to as Ser-51 based on the posttranslational cleavage of the amino-terminal methionine in the mature human α protein.) Interestingly, consensus cAMP-dependent protein kinase phosphorylation sites (26) are apparent in two different regions of both proteins. One is located at the same position in the yeast and human sequence between amino acid positions +88 and +91 (Arg-Arg-Val-Ser). The other, being present in the human sequence between amino acids +182 and +185 (Arg-Arg-Leu-Thr), shows partial homology to amino acid positions +175 to +178 (Lys-Arg-Leu-Thr) in the yeast sequence. No information exists as to whether these latter two sites are targets for phosphorylation either *in vivo* or *in vitro*.

Relationship of Sui2 to Yeast eIF-2α. Based on the sequence homology between the *SUI2* and the human eIF-2α gene products, a simple interpretation is that *SUI2* encodes the α subunit of yeast eIF-2. To test this, we partially purified eIF-2 from wild-type yeast cells (5) and analyzed this preparation by Western blot (17) using antiserum directed against the Sui2 gene product. This eIF-2 preparation represents the 400 mM phosphate fraction that was derived from salt-washed ribosomal pellets chromatographed on hydroxyapatite and has been shown by workers in our laboratory to contain eIF-2 activity (GTP-dependent binding of initiator tRNA) and the β subunit of eIF-2 (5). As shown in Fig. 5*A* (lane 1), antisera directed against Sui2 cross-reacts in a specific fashion with a protein of apparent mass 37-kDa and copurifies with a 36-kDa protein that cross-reacts with antisera directed against the β subunit of eIF-2 (Fig. 5*A*, lane 2).

Given consensus phosphorylation sites noted in the *SUI2* sequence shared with the human α sequence and especially identity around Ser-52 that is believed to be a site phosphorylated in mammalian cells, we also tested whether the 37-kDa protein that cross-reacts with the anti-Sui2 can be identified as a phosphoprotein in yeast. A *SUI2*$^+$ strain was grown in the presence of [^{32}P]orthophosphate and immunoprecipitated using anti-Sui2. As shown in Fig. 5*B* (lane 1), the immunoprecipitable product corresponds to the apparent mass of Sui2 (37 kDa) and upon autoradiography (Fig. 5*B*, lane 2) this protein band is identified as a ^{32}P-labeled product.

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                                                                    Eco RI
                                                                    GAATTCAGTTCTACTGGGATGATAATATTACTGGATTTGGAAACGTTAGAAGCAGAGACGGTCAAGATTGAC

                                                                                                                    Pvu II
ATTTAAATAAAACAAGGAATAATTTCCACATAGATATGCAATTAAGTTTTATATGTAAAAGTGAGCATTCATCGTTCAGCTCAAAATACGTTTCTTGTCACAGCTGGTAGAAAAACTATA

AGCGTTTTTTCTTACCCGCAGTCGGAGAAAAATTTTTTCTTCGAAGAGGCGAAAAAGAGAAGAAGAGAAAGCACAAATCTGATGAAATAGTAGTATAAAATCGCATTTACAAATTTTCAA

CCATTGTTTATTTCCTAGGTCATTAAAGAGTAAAGTGCAATCTGTTTACTAATCAGTTTTTGTCTTCATATTTTTGTGTCTTTTCTGCTGCCTCACGCACCTTCTATAATACACCAAATA

1                                ▽                   ▼
ATGTCCACTTCTCATTGCAGATTTTATGAAAACAAATACCCAGAAATTGACGATATCGTCATGGTTAACGTCCAGCAGATTGCTGAAATGGGTGCTTATGTTAAATTGTTAGAATATGAC
MetSerThrSerHisCysArgPheTyrGluAsnLysTyrProGluIleAspAspIleValMetValAsnValGlnGlnIleAlaGluMetGlyAlaTyrValLysLeuLeuGluTyrAsp

41                                                                                                              Sal I
AACATTGAAGGTATGATTCTACTAAGTGAATTGTCCCGTAGACGTATTAGGTCAATCCAAAAATTAATTCGTGTTGGTAAAAATGATGTCGCCGTTGTTCTTCGTGTCGACAAAGAAAAA
AsnIleGluGlyMetIleLeuLeuSerGluLeuSerArgArgArgIleArgSerIleGlnLysLeuIleArgValGlyLysAsnAspValAlaValValLeuArgValAspLysGluLys

81
GGTTATATTGATTTGTCCAAACGTCGTGTTTCTTCTGAAGATATCATTAAATGTGAAGAAAAATACCAAAAATCTAAGACTGTTCATTCCATTTTAAGATACTGTGCCGAAAAATTCCAA
GlyTyrIleAspLeuSerLysArgArgValSerSerGluAspIleIleLysCysGluGluLysTyrGlnLysSerLysThrValHisSerIleLeuArgTyrCysAlaGluLysPheGln

121                                                     Hind III
ATCCCTTTGGAAGAACTATATAAGACCATTGCTTGGCCATTAAGTCGAAAATTTGGTCACGCTTACGAAGCTTTCAAACTATCCATCATTGACGAAACTGTTTGGGAAGGTATTGAACCG
IleProLeuGluGluLeuTyrLysThrIleAlaTrpProLeuSerArgLysPheGlyHisAlaTyrGluAlaPheLysLeuSerIleIleAspGluThrValTrpGluGlyIleGluPro

161                                                                                                             Cla I
CCATCAAAAGATGTTTTAGATGAATTAAAGAACTATATCTCCAAGAGATTAACACCACAAGCTGTAAAGATTAGAGCCGATGTTGAAGTGTCTTGTTTTAGTTACGAAGGTATCGATGCC
ProSerLysAspValLeuAspGluLeuLysAsnTyrIleSerLysArgLeuThrProGlnAlaValLysIleArgAlaAspValGluValSerCysPheSerTyrGluGlyIleAspAla

201              Pvu II
ATTAAAGACGCATTAAAATCAGCTGAAGACATGTCCACAGAACAAATGCAAGTTAAAGTTAAATTAGTCGCCGCCCCATTATATGTTTTGACCACCCAAGCCTTGGATAAGCAAAAAGGT
IleLysAspAlaLeuLysSerAlaGluAspMetSerThrGluGlnMetGlnValLysValLysLeuValAlaAlaProLeuTyrValLeuThrThrGlnAlaLeuAspLysGlnLysGly

241
ATTGAACAACTGGAAAGCGCTATTGAAAAAATTACAGAGGTTATTACAAAATACGGCGGTGTTTGCAACATTACCATGCCACCAAAGGCTGTCACTGCTACTGAAGACGCTGAGTTACAA
IleGluGlnLeuGluSerAlaIleGluLysIleThrGluValIleThrLysTyrGlyGlyValCysAsnIleThrMetProProLysAlaValThrAlaThrGluAspAlaGluLeuGln

281                              Bgl II
GCTCTATTAGAAAGCAAAGAATTAGATAATAGATCTGACTCTGAAGACGATGAGGATGAGTCAGACGACGAGTAATCATTGCCGCGCCTAATTTTTCTAGGTGTTTTCAAGTGTCATACT
AlaLeuLeuGluSerLysGluLeuAspAsnArgSerAspSerGluAspAspGluAspGluSerAspAspGluTer

                        SnaB I
GTTTTAGAAAATTTTGTATAGAACAAATACGTATATCCTGCCATATCATATTCTTTGCAATATACACCTTGTACATTTGGCTATTATAAATATTACAATCCATTTAATCATAATCAAAAT
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FIG. 3. DNA sequence of the wild-type *SUI2* gene product. The DNA sequence corresponding to the 304-amino acid sequence of the *SUI2* gene product numbered from the ATG (+1) was confirmed for both DNA strands by either the Maxam and Gilbert (21) or dideoxynucleotide chain-termination (20) method (see Fig. 1 for sequencing strategies). The open and solid triangles indicate the nucleotide changed in the mutant *sui2-1* [*C*CA (Pro) → *T*CA (Ser)] and *sui2-2* [*G*TC (Val) → *T*CC (Phe)] that alter amino acid positions +14 and +20, respectively.

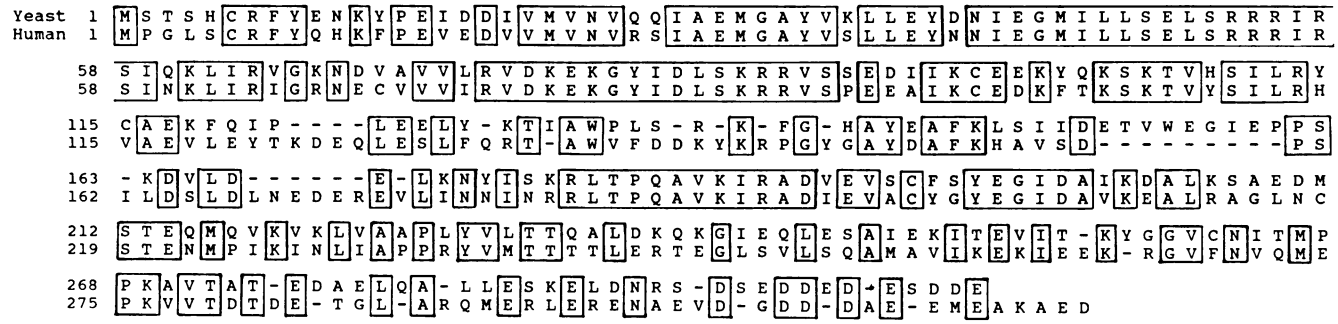

FIG. 4. Comparison of the wild-type yeast *SUI2* and human eIF-2α amino acid sequences (single-letter code). The 304-amino acid sequence for the Sui2 protein is derived from the *SUI2*$^+$ DNA sequence in Fig. 3 and is compared to the 315-amino acid sequence derived from a human cDNA sequence reported to encode the α-subunit of eIF-2 (7). The amino acid positions are numbered relative to the methionine (+1) encoded by the AUG start codon in both genes. The two proteins are aligned, introducing gaps within the sequences to maximize the homology. Identical amino acids in the human and yeast sequences are boxed.

Similar studies with *sui2* suppressor strains indicate that the 37-kDa protein and the corresponding ^{32}P-labeled product are greatly reduced and suggests that mutations in *sui2* confer instability to the 37-kDa protein (data not shown). In addition, a partially purified preparation of eIF-2 from the *sui2-1* suppressor strain shows defects in ternary complex formation, lacking any detectable GTP-dependent binding of initiator tRNA$_i^{met}$. Therefore, based on the high degree of amino acid conservation observed between *SUI2* and the human eIF-2α sequences, predicted biochemical characteristics and eIF-2 defects conferred by *sui2* mutations, we conclude that *SUI2* encodes the α subunit of the yeast eIF-2 complex.

Mechanism of Suppression. We have previously demonstrated that a *SUI3* suppressor gene that encodes a mutant β subunit of eIF-2 restores translation initiation to *his4* and *his4-lacZ* initiator codon mutant strains by affording initiation at a UUG codon present at amino acid position +4 in the early *his4* coding region (5). To determine whether mutations in the α subunit restore His4 expression by a similar mechanism, we purified the gene product produced from the mutant *his4-lacZ* mRNA in a *sui2-1* revertant strain and sequenced the amino terminus. The yields of phenylthiohydantoin (PTH)-derivatized amino acids from the first five cycles of the protein sequence when an AUU initiator codon mutation is present in the *his4-lacZ* message are presented as a bar graph in Fig. 6. The predominant sequence, Pro-Ile-Leu-Pro-Leu, matches the *HIS4* coding region beginning at the fourth codon 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 *SUI3* suppression (5). Therefore, the effects of mutating the α subunit in yeast are qualitatively similar to mutating the β subunit of eIF-2, presumably by allowing the standard methionine initiator tRNA to initiate translation at a UUG codon inserting methionine that is cleaved posttranslationally (5).

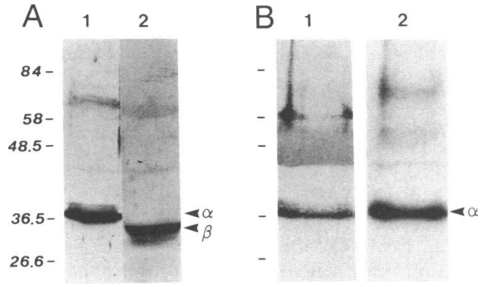

FIG. 5. Western blot and phosphorylation analyses. eIF-2 was partially purified from the *SUI2* wild-type strain as described (5) and electrophoresed on 10% SDS/polyacrylamide gel containing 6 M urea (19), transferred to nylon, blocked, and then probed with rabbit antibodies (1:150) raised against TrpE-Sui2 produced in *E. coli*. Prestained molecular mass standards (kDa) were coelectrophoresed with each gel. Antigen–antibody complexes were visualized using a horseradish peroxidase conjugate system. Western blot analysis of yeast crude extracts demonstrates that the antibodies raised against the TrpE-Sui2 proteins cross-react with a 37-kDa protein that responds to an increase in *SUI2* gene dosage associated with the high copy *SUI2*$^+$ yeast strain BC-52 as opposed to the single copy *SUI2*$^+$ strain BC-36 (data not shown). Substitution of preimmune sera (1:100) did not reveal cross-reactivity (data not shown). (*A*) Lane 1, 400 mM phosphate fraction eluted from hydroxyapatite chromatography and probed with anti-Sui2. Lane 2, same as lane 1 except that anti-eIF-2β was used as the probe (5). (*B*) Crude yeast extracts derived from ^{32}P-labeled *SUI2*$^+$ yeast cells were immunoprecipitated with rabbit antibodies directed against the TrpE-Sui2 fusion protein. Lane 1, immunoprecipitable products were electrophoresed as described above and visualized by Western blot analysis using TrpE-Sui2 antibodies. Lane 2, autoradiograph of lane 1. α, eIF-2α subunit; β, eIF-2β subunit.

DISCUSSION

As previously stated, eIF-2 functions in the early steps of protein synthesis by binding tRNA and GTP to form a ternary complex, which in turn binds the 40S subunit and scans mRNA in search of a translational start site. Genetic studies in our laboratory suggest that the close association of the ternary complex with 40S up until the time of 80S complex formation reflects an additional function for eIF-2 at the time of start site selection. Namely, mutations at a zinc(II) finger motif in the *SUI3* suppressor gene, which encodes the β subunit of eIF-2 (5), and, as reported here, mutations in the α subunit encoded by *sui2* both confer the ability to the ribosome to no longer bypass a mutant initiator region at *his4* but now recognize and initiate at a UUG codon. However, eIF-2 is not the only component of the ternary complex that is important for the recognition of a start codon as we have also shown that an anticodon mutation in tRNA$_i^{met}$ can direct the ribosome to initiate at a complementary non-AUG codon in the *HIS4* message (28). Thus, our studies suggest that eIF-2 and tRNA$_i^{met}$ work in concert to establish the site of initiation. The fact that mutations in the amino terminus of the sui2 protein alter start site selection suggests that this region may be directly involved in mediating the recognition of an AUG codon. Whether these mutations define a region that interacts with nucleic acids, as suspected for mutations in the zinc(II) finger motif of β (5), or mutations in the α protein alter β function through a protein:protein interaction to arrive at non-AUG initiation events remains to be established.

Interestingly the yeast and human α sequences are highly conserved, especially in regions that are either believed to be phosphorylated or correspond to consensus target sites for a cAMP-dependent protein kinase. In mammalian cells, induced or increased phosphorylation of α in response to cellular stress (29, 30) is suspected to play a role in controlling

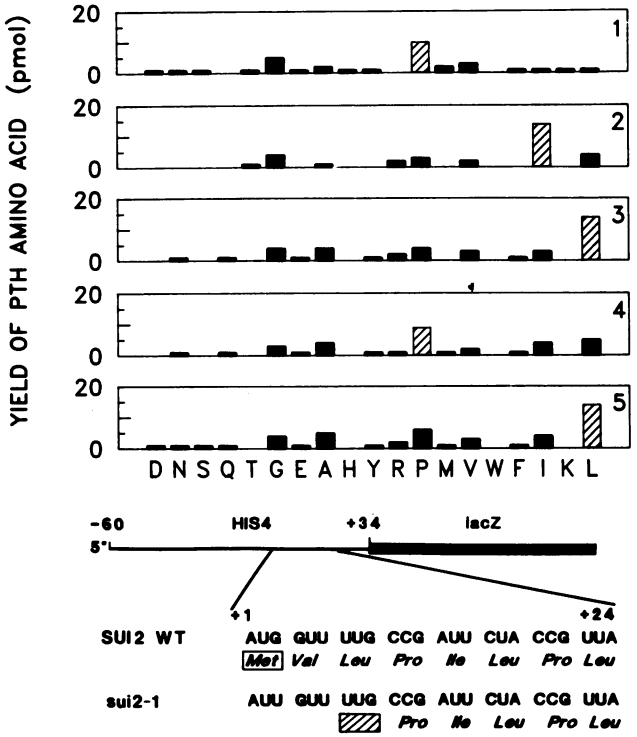

FIG. 6. Amino-terminal sequence analysis of His4 β-galactosidase product. The His4-β-galactosidase fusion protein synthesized from the *sui2-1* suppressor strain 117-8AR20 that contains an AUU initiator codon change in the *his4-lacZ* message was purified to homogeneity by a previously described purification scheme (5). Amino acid sequence analysis was determined by automated Edman degradation using an Applied Biosystems liquid-phase protein/peptide sequencer (model 477A) with an on-line PTH-amino acid analyzer (model 120A). The amino-terminal sequence ( ) is presented as a bar graph plotted as the total yield (pmol) of each PTH-amino acid derivative (single-letter amino acid designation) for the first five (1–5) sequencing cycles per His4 β-galactosidase product sequenced. The results are interpreted below the bar graph relative to the known amino-terminal sequence Val-Leu-Pro-Ile-Leu [methionine (boxed) is cleaved post-translationally] of the *HIS4*$^+$ wild-type (WT) protein (27).

rates of protein synthesis. The fact that putative phosphorylation sites are intact in the *SUI2* sequence as compared to the human eIF-2α sequence and anti-Sui2 identifies a phosphoprotein of predicted molecular mass (Figs. 4 and 5) suggests that similar mechanisms to control protein synthesis by phosphorylation of α (31, 32) may be functional in yeast and therefore amenable to genetic analysis.

Another important result of the *sui2* analysis is that it provides clarity with regard to the biochemical characteristics of the subunit composition of eIF-2 in yeast. A previous biochemical analysis of yeast eIF-2 reported that it is composed of three nonidentical subunits having apparent values of 31, 46.5, and 49 kDa on SDS/PAGE with the 31-kDa protein corresponding to the α subunit (18, 33). Based on a later study that analyzed the phosphorylation of the 31-kDa protein under different physiological conditions (18), it was concluded that the modification profile of this 31-kDa protein does not change in response to growing yeast under different physiological conditions, suggesting that phosphorylation of the eIF-2α subunit in yeast is not related to a mechanism of controlling the rate of protein synthesis. In contrast, we find that α and β comigrate on 10% SDS/polyacrylamide gels as apparent 36-kDa proteins (data not shown) and by using urea gels the α and β subunits can be separated as apparent 37- and 36-kDa proteins, respectively (Fig. 5*A*). In addition, upon following the reported purification of eIF-2 (33), we find little or no eIF-2 activity associated with this preparation, nor do any of the three proteins in this preparation cross-react with anti-Sui2 or anti-Sui3. Instead, our data do agree with another study showing that the α subunit is a 36-kDa protein (34). Therefore, in light of our observations, the control of protein synthesis by phosphorylation of α in yeast should be readdressed by more direct experimentation. This conclusion is especially relevant given the similarities between the *SUI2* and human α sequence in the regions of the protein that may be the target for phosphorylation.

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