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# Functional Elements in Initiation Factors 1, 1A, and 2β Discriminate against Poor AUG Context and Non-AUG Start Codons[▽]

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**Yeast eIF1 inhibits initiation at non-AUG triplets, but it was unknown whether it also discriminates against AUGs in suboptimal context. As in other eukaryotes, the yeast gene encoding eIF1 (*SUI1*) contains an AUG in poor context, which could underlie translational autoregulation. Previously, eIF1 mutations were identified that increase initiation at UUG codons (Sui$^-$ phenotype), and we obtained mutations with the opposite phenotype of suppressing UUG initiation (Ssu$^-$ phenotype). Remarkably, Sui$^-$ mutations in eukaryotic translation initiation factor 1 (eIF1), eIF1A, and eIF2β all increase *SUI1* expression in a manner diminished by introducing the optimal context at the *SUI1* AUG, whereas Ssu$^-$ mutations in eIF1 and eIF1A decrease *SUI1* expression with the native, but not optimal, context present. Therefore, discrimination against weak context depends on specific residues in eIFs 1, 1A, and 2β that also impede selection of non-AUGs, suggesting that context nucleotides and AUG act coordinately to stabilize the preinitiation complex. Although eIF1 autoregulates by discriminating against poor context in yeast and mammals, this mechanism does not prevent eIF1 overproduction in yeast, accounting for the hyperaccuracy phenotype afforded by *SUI1* overexpression.**

Bacterial translation initiation factor 3 (IF3) promotes the fidelity of initiation at AUG codons by discriminating against non-AUG triplets as start sites (17, 26, 40, 45). This discriminatory function forms the basis for IF3's ability to negatively autoregulate translation of its mRNA, which initiates with an AUU start codon (5, 6). IF3 also destabilizes initiation complexes formed on AUG codons at the 5′ ends of leaderless mRNAs (47), which lack the Shine-Dalgarno sequence that stabilizes mRNA association with the small (30S) ribosomal subunit at the AUG codon.

In eukaryotes, the 43S preinitiation complex (PIC), harboring the eIF2-GTP-Met-tRNA$_i^{Met}$ ternary complex (TC) and various other eIFs, attaches to the capped 5′ end of the mRNA and identifies the AUG codon by scanning the mRNA leader base-by-base for complementarity with the anticodon of Met-tRNA$_i^{Met}$. Efficient initiation is influenced by the sequence immediately upstream from the AUG, but it is unclear how this sequence context is recognized or regulates AUG selection (20, 36). The functional counterpart of IF3 in eukaryotes appears to be eIF1 (Sui1 in yeast). eIF1 and IF3 occupy analogous locations on the platform of the small ribosomal subunit (11, 27, 38) and, similar to IF3, eIF1 blocks formation of stable 48S PICs at near-cognate start codons (20, 36). eIF1/Sui1 and eIF1A (Tif11 in yeast) cooperate to promote an open conformation of the 40S subunit (34) thought to be conducive to scanning (35), and eIF1 also blocks the final step of GTP hydrolysis by the TC, the release of $P_i$ from eIF2-GDP-$P_i$, until an AUG enters the P site (1). AUG recognition triggers dissociation of eIF1 from the 40S subunit (30), enabling $P_i$ release and stabilizing a closed conformation of the 40S subunit that is incompatible with scanning.

Similar to the effects of IF3 mutations in bacteria, hypomorphic mutations in yeast eIF1 (an essential protein) increase initiation from UUG codons *in vivo* (12, 49), i.e., the Sui$^-$ phenotype, whereas overexpressing wild-type (WT) eIF1 suppresses UUG initiation in mutants with Sui$^-$ substitutions in other initiation factors, i.e., the Ssu$^-$ phenotype (41, 48). Interestingly, Sui$^-$ mutations in eIF1 generally weaken its affinity for the 40S and enable inappropriate eIF1 dissociation and $P_i$ release from eIF2-GDP-$P_i$ at non-AUG codons. Furthermore, a mutation in the N-terminal tail (NTT) of eIF1A (*17-21*) that suppresses UUG initiation *in vivo* retards eIF1 dissociation on start codon recognition (8). Thus, the rate of eIF1 dissociation and $P_i$ release are critical determinants of AUG recognition. Suppression of the increased UUG initiation frequency in Sui$^-$ mutants by eIF1 overexpression can be understood as the consequence of impeding rearrangement of the PIC from the eIF1-bound, scanning conformation to the closed, scanning-arrested state lacking eIF1 in the absence of an AUG-anticodon match, with a UUG in the P site.

It was reported recently that the genes encoding eIF1 in diverse eukaryotes contain an AUG context that deviates significantly from that found at highly expressed genes (22, 31). In particular, eIF1 genes generally contain pyrimidines rather than purines at the −3 position, shown by Kozak to be the most critical contextual determinant of AUG selection in mammals (24). Using a reconstituted *in vitro* system, it was demonstrated that mammalian eIF1 can discriminate against AUGs in suboptimal context in addition to preventing recognition of non-AUG codons (35), and it was envisioned that context nucleotides help to stabilize the closed conformation of the PIC that is competent for start codon recognition (37). As

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first proposed by Ivanov et al. (22), its ability to discriminate against poor AUG context would allow mammalian eIF1 to negatively autoregulate translation by discriminating against the poor context of its start codon and, while our work was under way, these researchers provided strong evidence that this autoregulatory mechanism indeed operates in mammalian cells.

Adenines at positions −3 to −1 relative to AUG are highly preferred among genes in *Saccharomyces* species (44), and there is evidence that $A_{-3}$-$A_{-2}$-$(A/G)_{-1}$-AUG is the optimal context for initiation in *Saccharomyces cerevisiae* (7). *Saccharomyces* species resemble other eukaryotes in displaying poor context for the AUG codon at the eIF1 gene (*SUI1*), $C_{-3}$-$G_{-2}$-$U_{-1}$-AUG, which matches at −3 and −2 a highly unfavorable sequence context identified in *S. cerevisiae*, of $C_{-3}$-$G_{-2}$-$C_{-1}$-AUG (7). However, it was unknown whether eIF1 autoregulates translation in yeast, or whether other eIFs participate in evaluating AUG context in any eukaryotic cells. In this report, we show that $Sui^-$ and $Ssu^-$ substitutions in eIF1/Sui1 suppress and exacerbate, respectively, the deleterious effect of poor AUG context on eIF1 expression. We extend this finding to include $Sui^-$ and $Ssu^-$ substitutions in eIF1A and eIF2β (Sui3 in yeast) and a suboptimal context that is even less functional than that present at native *SUI1*. These findings indicate that eIF1 autoregulates translation in yeast and that discrimination against poor context *in vivo* depends on specific domains and residues in eIF1, eIF1A, and eIF2β that also function in stringent selection of AUG codons. This strongly supports the notion that a favorable context and AUG triplet cooperate to promote the rearrangement from an open, scanning conformation to a closed, initiation-competent state of the PIC.

## MATERIALS AND METHODS

**Yeast strain constructions.** To generate strains PMY30 through PMY51 and PMY98, strain JCY03 [*MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301* (*ACG*) *sui1*Δ*::hisG* p1200 (sc *URA3 SUI1*)] was transformed to $Leu^+$ with single-copy (sc) or high-copy-number (hc) *LEU2* plasmids harboring the appropriate *SUI1* alleles on SC-L medium, and the resident *SUI1*$^+$ *URA3* plasmid (p1200) was evicted by selecting for growth on 5-fluoorotic acid (5-FOA) medium. The plasmids and yeast strains discussed in the present study are listed in Tables 1 and 2, respectively.

Strains PMY01, PMY02, and PMY03, in which *TIF5*, *SUI3* or *TIF11*, respectively, are under the control of the *GAL1* promoter, were generated from JCY03 by the one-step PCR strategy (28) selecting for resistance to kanamycin on rich medium containing galactose as carbon source (YPGal). Integration of the *kanMX:*$P_{GAL1}$ promoter cassette at the correct chromosomal location was verified by PCR analysis of genomic DNA using the appropriate primers.

Strain PMY01 was transformed with an sc *TRP1* plasmid containing *SUI5* (p4281/YCpTIF5-G31R-W) on synthetic complete medium containing galactose as a carbon source and lacking tryptophan (SGal-W) to generate PMY04. To obtain strains PMY52 through PMY59, PMY04 was transformed with sc or hc *LEU2* plasmids harboring the appropriate *SUI1* alleles on SGal lacking leucine and tryptophan (SGal-LW), and p1200 was evicted on 5-FOA medium containing galactose as a carbon source (5-FOA/Gal).

Myc-tagging of *HIS4* in strain PMY16 was constructed as follows. A PCR fragment containing a portion of *HIS4* from 340 bp upstream to 437 bp downstream of the ATG was amplified from genomic DNA of H466 using the primers PM-18 (5′-GAGCATTGCGATACGATGGG-3′) and PM-19 (5′-CGGTCTGT ACGTACTTCACC-3′). The PCR product was used to transform strain JCY04 to $His^+$ on SC lacking uracil and histidine (SC-UH), and replacement of *his4-301-myc*$_{10}$ with *HIS4-myc*$_{10}$ was verified by PCR analysis of chromosomal DNA and sequencing using the appropriate primers.

To obtain strains JCY806 through JCY810, and PMY60 through PMY62, strain JCY04 was cotransformed with a sc *TRP1* plasmid containing *SUI5* (p4281/YCpTIF5-G31R-W) and sc or hc *LEU2* plasmids harboring the appropriate *SUI1* alleles on SC-LW medium, and p1200 was evicted on 5-FOA medium. The same procedure was followed to generate PMY63 through PMY70 except beginning with PMY16 rather than JCY04.

To produce strains PMY71 through PMY78, JCY03 was cotransformed with an sc *TRP1* plasmid containing *SUI3-2* (p4280/YCpSUI3-S264Y-W) and sc or hc *LEU2* plasmids harboring the appropriate *SUI1* alleles on SC-LW medium, and p1200 was evicted on 5-FOA medium.

Strains PMY79 through PMY82 were constructed by cotransforming PMY02 with an sc *LEU2* plasmid containing *SUI1* (pJCB101) or *SUI1-opt* (pPMB10) and with an sc *TRP1* plasmid containing *SUI3* (p4450) or *SUI3-2* (p4280/YCpSUI3-S264Y-W), on SGal-LW, and p1200 was evicted on 5-FOA/Gal medium.

Strains PMY83 through PMY88 were generated by cotransforming PMY03 with an sc *TRP1* plasmid harboring *SUI1* (pPMB21) or *SUI1-opt* (pPMB22) and with an sc *LEU2* plasmid containing *TIF11* (pDSO9), *tif11-17-21* (p4552), or *tif11-SE*$_1$*, *SE*$_2$**+F131* (pAS23) on SGal-LW, and p1200 was evicted on 5-FOA/Gal medium.

Strains PMY89 through PMY93 were produced by cotransforming PMY01 with an sc *LEU2* plasmid containing *SUI1* (pJCB101) or *SUI1-opt* (pPMB10) and with an sc *TRP1* plasmid containing *TIF5-FL* (p4119/TIF5-FL TRP1) or *SUI5* (p4281/YCpTIF5-G31R-W), or with empty *TRP1* vector YCplac22, on SGal-LW, and p1200 was evicted on 5-FOA/Gal medium.

**Plasmid constructions.** Plasmid pJCB101 was constructed by cloning a 0.9-kb BssHII-SacII fragment containing *SUI1* from p1200 into similarly digested pCFB03. The construction of plasmids pPMB01 through pPMB08 is described in the next section.

pPMB30 was created by inserting a 1.6-kb HindIII-SacI fragment containing *sui1-L96P* from pPMB03 into the corresponding sites of YCplac181.

To construct pPMB09, containing *sui1-E48V,L51F*, the QuikChange site-directed mutagenesis system (Stratagene) was used with the primers PM-74 (5′-CCCAGAGGTATATGATTTTAAGAGAATTCTTAAGGTC-3′) and PM-75 (5′-GACCTTAAGAATTCTCTTAAAATCATATACCTCTGGG-3′), using pPMB05 as a template.

To introduce the optimal sequence context $A_{-3}$-$A_{-2}$-$A_{-1}$-AUG into the corresponding sc or hc plasmids containing either *SUI1* or different *sui1* mutant alleles, the QuikChange site-directed mutagenesis system (Stratagene) was used with the primers PM-181 (5′-TATAGCTGAAGCAAATAAAATGTCCATTG AGAATC-3′) and PM-182 (5′-GATTCTCAATGGACATTTTATTTGCTTCA GCTATA-3′).

To construct pPMB24, an 812-bp *SUI1* fragment containing 577 bp upstream and 212 bp downstream of the ATG was amplified by PCR from pJCB101 using primers PM-169 (5′- GTGAGCTACGCGTCGACAGATCTGAATCTATTCT GGAC-3′) and PM-170 (5′- CGCGGATCCTTGACAATGTTACCATTACAT GC-3′) introducing a novel SalI site in the 5′ end and a BamHI site in the 3′ end of the fragment, digested with SalI and BamHI, and inserted between the corresponding sites in the *GCN4* plasmid p164, generating pPMB23. A BamHI fragment containing *lacZ* was excised from p180 and inserted into the BamHI site of pPMB23 to produce pPMB24, harboring a *SUI1-lacZ* fusion construct that includes the promoter region and first 215 bp of the *SUI1* open reading frame (ORF), the *lacZ* ORF, and *GCN4* sequences extending from the BamHI site through the 3′ untranslated region to the EcoRI site 3′ of *GCN4*.

pPMB25 and pPMB28 were constructed by cloning the same 812-bp SalI/BamHI fragment containing *SUI1* just described into pCR2.1-TOPO (Invitrogen). Site-directed mutagenesis was conducted with the QuikChange system by using primer pairs (i) PM-181 (5′-TATAGCTGAAGCAAATAAAATGTCCA TTGAGAATC-3′) and PM-182 (5′-GATTCTCAATGGACATTTTATTTGCT TCAGCTATA-3′) to introduce the $A_{-3}$-$A_{-2}$-$A_{-1}$-AUG context and (ii) PM-206 (5′-TATAGCTGAAGCAAATTTTATGTCCATTGAGAATC-3′) and PM-207 (5′-GATTCTCAATGGACATAAAATTTGCTTCAGCTATA-3′) to introduce the $U_{-3}$-$U_{-2}$-$U_{-1}$-AUG context. The resulting PCR products were digested with BamHI and SalI and cloned into p164, and the corresponding *SUI1-lacZ* fusions, pPMB25 and pPMB28, were generated as described above for pPMB24.

To construct pPMB26 and pPMB27, an ~4.1-kb SalI-KpnI fragment containing either *SUI1-lacZ* or *SUI1-opt-lacZ* from plasmids pPMB24 and pPMB25, respectively, was cloned between the corresponding sites of pHQ1303.

**Selection of mutant *SUI1* alleles.** Random mutagenesis of *SUI1* was conducted by error-prone PCR using the *SUI1 URA3* plasmid p1200 as a template with the primers JCO075 (5′-TGTACACTATGCATGCGCGT-3′) and JCO076 (5′-CC CCATGATAATGTACTCTCG-3′) and a GeneMorph II random mutagenesis kit (Stratagene). A 0.9-kb BssHII-SacII fragment from *His-SUI1* plasmid pCFB03 was replaced with the resulting PCR products and ~67,000 bacterial transformants were pooled. Plasmid DNA was prepared from the pool for

TABLE 1. Plasmids used in this study

| Plasmid | Description[a] | Source or reference |
|---|---|---|
| YCplac111 | sc *LEU2* cloning vector | 16 |
| YEplac181 | hc *LEU2* cloning vector | 16 |
| YCplac22 | sc *TRP1* cloning vector | 16 |
| p1200 | sc *URA3 SUI1* in YCp50 | 49 |
| pCFB03 | sc *LEU2 His-SUI1* in YCplac111 | 8 |
| pJCB101 | sc *LEU2 SUI1* in YCplac111 | This study |
| pCFB04 | hc *LEU2 SUI1* in YEplac181 | 8 |
| pPMB01 | sc *LEU2 sui1-K56E* in YCplac111 | This study |
| pPMB02 | sc *LEU2 sui1-K60E* in YCplac111 | This study |
| pPMB03 | sc *LEU2 sui1-L96P* in YCplac111 | This study |
| pPMB30 | hc *LEU2 sui1-L96P* in YEplac181 | This study |
| p367 | sc *URA3 HIS4*(*ATG*)*-lacZ* | 13 |
| p391 | sc *URA3 HIS4*(*TTG*)*-lacZ* | 13 |
| p4281/YCpTIF5-G31R-W | sc *TRP1 TIF5-G31R* in YCplac22 | 48 |
| p4280/YCpSUI3-S264Y-W | sc *TRP1 SUI3-S264Y* in YCplac22 | 48 |
| pPMB04 | sc *LEU2 sui1-T15A* in YCplac111 | This study |
| pPMB05 | sc *LEU2 sui1-E48V* in YCplac111 | This study |
| pPMB06 | sc *LEU2 sui1-L51F* in YCplac111 | This study |
| pPMB07 | sc *LEU2 sui1-D61G* in YCplac111 | This study |
| pPMB08 | sc *LEU2 sui1-Q84H* in YCplac111 | This study |
| pPMB09 | sc *LEU2 sui1-E48V,L51F* in YCplac111 | This study |
| pPMB10 | sc *LEU2 SUI1-opt* in YCplac111 | This study |
| pPMB11 | hc *LEU2 SUI1-opt* in YEplac181 | This study |
| pPMB12 | sc *LEU2 sui1-K56E-opt* in YCplac111 | This study |
| pPMB13 | sc *LEU2 sui1-K60E-opt* in YCplac111 | This study |
| pPMB14 | sc *LEU2 sui1-L96P-opt* in YCplac111 | This study |
| pPMB15 | sc *LEU2 sui1-T15A-opt* in YCplac111 | This study |
| pPMB16 | sc *LEU2 sui1-E48V-opt* in YCplac111 | This study |
| pPMB17 | sc *LEU2 sui1-L51F-opt* in YCplac111 | This study |
| pPMB18 | sc *LEU2 sui1-D61G-opt* in YCplac111 | This study |
| pPMB19 | sc *LEU2 sui1-Q84H-opt* in YCplac111 | This study |
| pPMB20 | sc *LEU2 sui1-E48V,L51F-opt* in YCplac111 | This study |
| pPMB21 | sc *TRP1 SUI1* in YCplac22 | This study |
| pPMB22 | sc *TRP1 SUI1-opt* in YCplac22 | This study |
| p4450 | sc *TRP1 SUI3* in YCplac22 | C. Fekete |
| pDSO9 | sc *LEU2 TIF11* in YCplac111 | 9 |
| p4552 | sc *LEU2 TIF11-NDSDG*$_{17\text{-}21}$AAAAA *in YCplac111* | 14 |
| pAS23 | sc *LEU2 TIF11-FGFESDE*$_{121\text{-}127}$*AAAAAAA,FEFGN*$_{131\text{-}135}$*FAAAA* in YCplac111 | 41 |
| p4119/TIF5-FL TRP1 | sc *TRP1 TIF5-FL* in YCplac22 | K. Asano |
| p164 | sc *URA3 GCN4 in* YCp50 | 19 |
| p180 | sc *URA3 GCN4-lacZ* in YCp50 | 19 |
| pPMB23 | sc *URA3 SUI1-GCN4* in p164 | This study |
| pPMB24 | sc *URA3 SUI1-lacZ* in p164 | This study |
| pPMB25 | sc *URA3 SUI1-opt-lacZ* in p164 | This study |
| pHQ1303 | hc *URA3 GCN4 in* YEplac195 | 50 |
| pPMB26 | hc *URA3 SUI1-lacZ* in pHQ1303 | This study |
| pPMB27 | hc*URA3 SUI1-opt-lacZ* in pHQ1303 | This study |
| pPMB28 | sc *URA3 SUI1*$_{UUU}$*-lacZ* in p164 | This study |

[a] hc, high copy number; sc, single copy.

screening in the appropriate yeast strains. To identify Sui$^-$ alleles, Leu$^+$ yeast transformants of strain JCY03 were selected on SC-L and pooled, and dilutions were plated on SC-L supplemented with 5-FOA (to evict p1200) and 0.0015 mM histidine. Transformants with His$^+$ phenotype were colony purified, and the resident plasmids were isolated.

Ssu$^-$ mutations were identified as suppressors of the lethality of *SUI5*, as follows. Leu$^+$ Trp$^+$ transformants of strain PMY04 were selected on SGal-LW and pooled, and dilutions were plated on SC-LW supplemented with 5-FOA. Transformants that grew in the presence of glucose were colony purified, and the resident plasmids were isolated.

For each selection, the ability of the purified plasmids to confer the relevant phenotypes was verified after transformation of the same yeast strain used to screen the mutant library, and the complete DNA sequence of the 0.9-kb BssHII-SacII fragment was determined to identify the mutations present in *SUI1*. In most cases, more than one mutation was present either in the promoter or in the *SUI1* coding region. In identify the mutations that confer the relevant phenotypes, site-directed mutagenesis was conducted to generate *SUI1* alleles with single mutations using the QuikChange system and the appropriate primers to generate the following plasmids: (i) pPMB01, primers PM-43 (5′-AAGAGAATTCTTGAGGTCCTAAAGA-3′) and PM-44 (5′-TCTTTAGGACCTCAAGAATTCTCTT-3′); (ii) pPMB02, primers PM-47 (5′-CTTAAGGTCCTAAAGGAGGACTTTGCATG-3′) and PM-48 (5′-CATGCAAAGTCCTCCTTTAGGACCTTAAG-3′); (iii) pPMB03, primers PM-138 (5′-GAATTTATGATCTCCCAACCGGGATTGCAAAAGAAG-3′) and PM-139 (5′-CTTCTTTTGCAATCCCGGTTGGGAGATCATAAATTC-3′); (iv) pPMB04, primers PM-23 (5′-CCTTTCGCCGACGCAGGAGACGACG-3′) and PM-24 (5′-CGTCGTCTCCTGCGTCGGCGAAAGG-3′); (v) pPMB05, primers PM-25 (5′-GCAAGGTGTCCCAGAGGTATATGATTTAAAGAGAA-3′) and PM-26 (5′-TTCTCTTTAAATCATATACCTCTGGGACACCTTGC-3′); (vi) pPMB06, primers PM-33 (5′-CCCAGAGGAATATGATTTTAAGAGAATTCTTAAGGTC-3′ and PM-34 (5′-GACCTTAAGAATTCTCTTAAAATCATATTCCTCTGGG-3′); (vii) pPMB07, primers PM-132 (5′-GTCCTAAAGAAGGGCTTTGCATGTAATG-3′) and PM-133(5′-CATTACATGCAAAGCCCTTCTTTAGGAC-3′); and (viii) pPMB08, primers PM-27 (5′-CAGTTGCAGGGTGACCATAGAGCAA

AGGTTTGC-3′) and PM-28 (5′-GCAAACCTTTGCTCTATGGTCACCCTG CAACTG-3′).

**Biochemical assays.** Assays of β-galactosidase activity in whole-cell extracts (WCEs) were performed as described previously (32). For Western analysis, WCEs were prepared by trichloroacetic acid extraction as previously described (39), and immunoblot analysis was conducted as described previously (33) with antibodies against eIF1 (48), eIF2Bε/Gcd6 (4) or myc epitope (Sigma). Enhanced chemiluminescence or the Odyssey infrared imaging system (Li-Cor) was used to visualize immune complexes, and signal intensities were quantified by densitometry using NIH ImageJ software or with the Odyssey application software, respectively.

For Northern analysis, RNA was extracted as previously described (42), resolved by electrophoresis in 1.2% agarose–4% formaldehyde gels, blotted onto positively charged nylon membranes (Roche), and immobilized with a UV Stratalinker 2400 (Stratagene). The blots were probed with a 1.6-kb HindIII-SacI fragment from plasmid pJCB101 containing the entire *SUI1* ORF or with a 6.7-kb HindIII fragment containing the *PYK1* coding sequence (encoding pyruvate kinase), radiolabeled using a random primed DNA labeling kit from Roche.

## RESULTS

***SUI1* Sui$^-$ mutations increase *SUI1* expression by suppressing poor AUG context.** We recently conducted a random mutagenesis of *SUI1*, the structural gene for eIF1/Sui1, in an effort to saturate single amino acid replacements in eIF1 that would evoke strong Sui$^-$ phenotypes, increasing initiation at the third (UUG) codon in the mutant *his4-301* mRNA lacking an AUG start codon. Such mutations restore the ability to grow on medium lacking histidine or containing only 1% of the normal histidine supplement (−His medium), conferring a His$^+$ phenotype on *his4-301* cells. The mutant *SUI1* alleles were introduced on a *LEU2* plasmid into a *his4-301 sui1*Δ strain harboring WT *SUI1* on a *URA3* plasmid, and the latter *SUI1*$^+$ plasmid was evicted by counterselection on medium containing 5-FOA (3). Among the *sui1* alleles that conferred a His$^+$ phenotype, we chose three for detailed analysis, including two that generate Glu substitutions of Lys-56 or Lys-60 in helix α1 of eIF1, and the third producing a Pro substitution for Leu-96 in helix α2 (15). These mutant alleles are designated below as *K56E*, *K60E*, and *L96P*, respectively. As shown in Fig. 1A, compared to the *SUI1*$^+$ strain, all three *sui1* mutants exhibit increased growth on −His medium despite diminished growth on histidine-replete medium (+His), and the His$^+$ phenotype is most pronounced for *L96P* (Fig. 1A). These mutants also display an increased frequency of UUG initiation, detected by assaying matched *HIS4-lacZ* fusions with AUG or UUG start codons. The UUG/AUG initiation ratio was increased by a factor of ~5 to ~18 above the WT level, with the greatest increase observed for *L96P* (Fig. 1B), confirming that all three strains are *bona fide* Sui$^-$ mutants.

Because the Sui$^-$ phenotype generally results from a reduction in eIF1 function (8, 48, 49), it was important to demonstrate that the Sui$^-$ substitutions reduce eIF1 activity rather than its expression. Western analysis of WCEs revealed that the *sui1* mutants actually contain ~5- to ~12-fold higher than WT levels of eIF1 (normalized to eIF2Bε subunit Gcd6), with the largest increase observed for the strongest Sui$^-$ mutant (*L96P*) (Fig. 1C).

The fact that eIF1 expression is elevated by these Sui$^-$ mutations is consistent with the possibility that they increase translation initiation from the *SUI1* AUG codon by overcoming its poor sequence context. If so, the increased eIF1 expression should be dampened by replacing the poor context with the optimal context for initiation in yeast. As expected, replacing the poor context of the *SUI1* start codon ($C_{-3}$-$G_{-2}$-$U_{-1}$-AUG) with the optimal context ($A_{-3}$-$A_{-2}$-$A_{-1}$-AUG), in the *SUI1-opt* allele, increased the eIF1 level in WT cells by a factor of 2.7 (cf. the WT lanes in Fig. 1C and D and the Opt/WT ratio for WT in Fig. 1E). Notably, introducing the optimal context dampened by a factor of 2 to 3 the increase in eIF1 expression conferred by the Sui$^-$ substitutions. For example, *L96P* increased expression of *SUI1-opt* by a factor of 4.4 (Fig. 1D, Norm. eIF1/Gcd6) compared to the 12-fold increase observed for *SUI1* alleles with the WT context (Fig. 1C, Rel. eIF1/Gcd6). Moreover, the Sui$^-$ substitutions reduced or completely masked the stimulatory effect of introducing the optimal context on eIF1 expression, conferring Opt/WT ratios close to unity (Fig. 1E).

Importantly, the *sui1-L96P* allele on a high-copy (hc) plasmid produced a substantially higher level of eIF1 than that given by the *sui1-opt-L96P* allele in single copy (sc) (Fig. 1F, lanes 5 and 6 versus lanes 3 and 4), indicating that the lack of an increase in eIF1-L96P expression conferred by the optimal context (Fig. 1F, lanes 3 and 4 versus lanes 1 and 2) does not reflect saturation of Western signals achieved with anti-eIF1 antibodies or an unknown posttranslational mechanism that would block eIF1 overexpression. Furthermore, the fact that introducing the optimal AUG context dampens the effects of the Sui$^-$ mutations (Fig. 1E) makes it unlikely that they increase *SUI1* transcription or stability of *SUI1* mRNA as the means of increasing eIF1 expression. Together, these results support the idea that eIF1 Sui$^-$ mutations overcome the deleterious effect of poor context at the *SUI1* AUG codon.

To provide independent evidence that eIF1 Sui$^-$ mutations suppress poor AUG context and do not increase *SUI1* expression by stabilizing *SUI1* mRNA, we examined their effects in *trans* on expression of *SUI1-lacZ* fusions containing the native, poor context or the optimal context described above (*SUI1-opt-lacZ*). Consistent with the Western analysis in Fig. 1C and D, expression of β-galactosidase from *SUI1-opt-lacZ* was 2-fold higher than that of *SUI1-lacZ* in WT cells (Fig. 1G, WT), reflecting the stimulatory effect of optimal context. Moreover, the *sui1* alleles increased expression of the WT fusion by a factor of 2.4 to 2.8 (Fig. 1G, Rel. *SUI1-lacZ*) but produced increases of only 1.2- to 1.5-fold for the *SUI1-opt-lacZ* construct (Fig. 1G, Rel. *SUI1-opt-lacZ*). Importantly, introducing the optimal context increased β-galactosidase expression by factors of only 1.2 or less in the Sui$^-$ mutants compared to the 2-fold increase in WT cells (Fig. 1G, *SUI1-opt-lacZ*/*SUI1-lacZ* ratios). Introducing *SUI1-opt-lacZ* on an hc plasmid into WT cells increased the β-galactosidase activity by ~8-fold (Fig. 1H), ruling out the possibility that fusion expression cannot substantially exceed that observed in the Sui$^-$ mutants. These findings support the conclusion that the eIF1 Sui$^-$ mutations increase *SUI1* expression by overcoming the deleterious effect of poor AUG context on translation initiation.

Finally, to provide direct evidence that the *sui1* mutations increase translational efficiency, we compared their effects on the level of *SUI1* mRNA, measured by Northern analysis, and the level of eIF1 measured by Western analysis. Although the *K60E* and *L96P* mutations conferred significant increases in *SUI1* mRNA abundance (Fig. 2, *SUI1* alleles, Norm. *SUI1*/

TABLE 2. Yeast strains used in this study

| Strain | Genotype[a] | Source or reference |
|---|---|---|
| JCY03 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* p1200 (sc *URA3 SUI1*) | 8 |
| PMY30 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pJCB101 (sc *LEU2 SUI1*) | This study |
| PMY31 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB01 (sc *LEU2 sui1-K56E*) | This study |
| PMY32 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB02 (sc *LEU2 sui1-K60E*) | This study |
| PMY33 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB03 (sc *LEU2 sui1-L96P*) | This study |
| PMY98 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB30 (hc *LEU2 sui1-L96P*) | This study |
| PMY34 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB04 (sc *LEU2 sui1-T15A*) | This study |
| PMY35 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB05 (sc *LEU2 sui1-E48V*) | This study |
| PMY36 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB06 (sc *LEU2 sui1-L51F*) | This study |
| PMY37 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB07 (sc *LEU2 sui1-D61G*) | This study |
| PMY38 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB08 (sc *LEU2 sui1-Q84H*) | This study |
| PMY39 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB09 (sc *LEU2 sui1-E48V,L51F*) | This study |
| PMY40 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pCFB04 (hc *LEU2 SUI1*) | This study |
| PMY41 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB10 (sc *LEU2 SUI1-opt*) | This study |
| PMY42 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB12 (sc *LEU2 sui1-K56E-opt*) | This study |
| PMY43 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB13 (sc *LEU2 sui1-K60E-opt*) | This study |
| PMY44 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB14 (sc *LEU2 sui1-L96P-opt*) | This study |
| PMY45 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB15 (sc *LEU2 sui1-T15A-opt*) | This study |
| PMY46 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB16 (sc *LEU2 sui1-E48V-opt*) | This study |
| PMY47 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB17 (sc *LEU2 sui1-L51F-opt*) | This study |
| PMY48 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB18 (sc *LEU2 sui1-D61G-opt*) | This study |
| PMY49 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB19 (sc *LEU2 sui1-Q84H-opt*) | This study |
| PMY50 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB20 (sc *LEU2 sui1-E48V,L51F-opt*) | This study |
| PMY51 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG* pPMB11 (hc *LEU2 SUI1-opt*) | This study |
| PMY01 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p1200 (sc *URA3 SUI1*) | This study |
| PMY02 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-SUI3* p1200 (sc *URA3 SUI1*) | This study |
| PMY03 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF11* p1200 (sc *URA3 SUI1*) | This study |
| PMY04 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p1200 (sc *URA3 SUI1*) p4281 (sc *TRP1 TIF5-G31R*) | This study |
| PMY52 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pJCB101 (sc *LEU2 SUI1*) | This study |
| PMY53 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pPMB04 (sc *LEU2 sui1-T15A*) | This study |
| PMY54 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pPMB05 (sc *LEU2 sui1-E48V*) | This study |
| PMY55 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pPMB06 (sc *LEU2 sui1-L51F*) | This study |
| PMY56 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pPMB07 (sc *LEU2 sui1-D61G*) | This study |
| PMY57 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP, TIF5-G31R*) pPMB08 (sc *LEU2 sui1-Q84H*) | This study |
| PMY58 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*AUU*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pPMB09 (sc *LEU2 sui1-E48V,L51F*) | This study |
| PMY59 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ::*hisG kanMX6:*$P_{GAL1}$*-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pCFB04 (hc *LEU2 SUI1*) | This study |
| JCY04 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p1200 (sc *URA3 SUI1*) | 33 |
| JCY806 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1 TIF5-G31R*) pJCB101 (sc *LEU2 SUI1*) | This study |
| JCY807 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1, TIF5-G31R*) pPMB04 (sc *LEU2 sui1-T15A*) | This study |
| JCY808 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB05 (sc *LEU2 sui1-E48V*) | This study |
| PMY60 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1, TIF5-G31R*) pPMB06 (sc *LEU2 sui1-L51F*) | This study |
| JCY809 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB07 (sc *LEU2 sui1-D61G*) | This study |
| JCY810 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::KanMX *sui1*Δ::*hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB08 (sc *LEU2 sui1-Q84H*) | This study |
| PMY61 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB09 (sc *LEU2 sui1-E48V,L51F*) | This study |
| PMY62 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*)-*myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1 TIF5-G31R*) pCFB04 (hc *LEU2 SUI1*) | This study |
| H466 | *MAT*α *his1-29 gcn2-101 gcn3-101 ino1 ura3-52* | A. Hinnebusch |
| PMY16 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc*$_{10}$::*KanMX sui1*Δ::*hisG* p1200 (sc *URA3 SUI1*) | This study |
| PMY63 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc*$_{10}$::*KanMX sui1*Δ::*hisG* p4281 (sc *TRP1 TIF5-G31R*) pJCB101 (sc *LEU2 SUI1*) | This study |

*Continued on following page*

TABLE 2—*Continued*

| Strain | Genotype[a] | Source or reference |
|---|---|---|
| PMY64 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc$_{10}$::KanMX sui1*Δ*::hisG* p4281 (sc *TRP1, TIF5-G31R*) pPMB04 (sc *LEU2 sui1-T15A*) | This study |
| PMY65 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc$_{10}$::KanMX sui1*Δ*::hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB05 (sc *LEU2 sui1-E48V*) | This study |
| PMY66 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc$_{10}$::KanMX sui1*Δ*::hisG* p4281 (sc *TRP1, TIF5-G31R*) pPMB06 (sc *LEU2 sui1-L51F*) | This study |
| PMY67 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc$_{10}$::KanMX sui1*Δ*::hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB07 (sc *LEU2 sui1-D61G*) | This study |
| PMY68 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc$_{10}$::KanMX sui1*Δ*::hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB08 (sc *LEU2 sui1-Q84H*) | This study |
| PMY69 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc$_{10}$::KanMX sui1*Δ*::hisG* p4281 (sc *TRP1 TIF5-G31R*) pPMB09 (sc *LEU2 sui1-E48V,L51F*) | This study |
| PMY70 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 HIS4-myc$_{10}$::KanMX sui1*Δ*::hisG* p4281 (sc *TRP1 TIF5-G31R*) pCFB04 (hc *LEU2 SUI1*) | This study |
| PMY71 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pJCB101(sc *LEU2 SUI1*) | This study |
| PMY72 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pPMB04 (sc *LEU2 sui1-T15A*) | This study |
| PMY73 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pPMB05 (sc *LEU2 sui1-E48V*) | This study |
| PMY74 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pPMB06 (sc *LEU2 sui1-L51F*) | This study |
| PMY75 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pPMB07 (sc *LEU2 sui1-D61G*) | This study |
| PMY76 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pPMB08 (sc *LEU2 sui1-Q84H*) | This study |
| PMY77 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pPMB09 (sc *LEU2 sui1-E48V,L51F*) | This study |
| PMY78 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG* p4280 (sc *TRP1 SUI3-2*) pCFB04 (hc *LEU2 SUI1*) | This study |
| PMY79 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-SUI3* p4450 (sc *TRP1 SUI3*) pJCB101 (sc *LEU2 SUI1*) | This study |
| PMY80 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-SUI3* p4450 (sc *TRP1 SUI3*) pPMB10 (sc *LEU2 SUI1-opt*) | This study |
| PMY81 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-303*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-SUI3* p4280 (sc *TRP1 SUI3-2*) pJCB101 (sc *LEU2 SUI1*) | This study |
| PMY82 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-SUI3* p4280 (sc *TRP1 SUI3-2*) pPMB10 (sc *LEU2 SUI1-opt*) | This study |
| PMY83 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF11* pDSO9 (sc *LEU2 TIF11*) pPMB21 (sc *TRP1 SUI1*) | This study |
| PMY84 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF11* pDSO9 (sc *LEU2 TIF11*) pPMB22 (sc *TRP1 SUI1-opt*) | This study |
| PMY85 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF11* p4552 (sc *LEU2 TIF11-NDSDG$_{17-21}$*AAAAA*) pPMB21 (sc* TRP1 SUI1*)* | This study |
| PMY86 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF11* p4552 (sc *LEU2 TIF11-NDSDG$_{17-21}$AAAAA*) pPMB22 (sc *TRP1 SUI1-opt*) | This study |
| PMY87 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF11* pAS23 (sc *LEU2 TIF11-FGFESDE$_{121-127}$AAAAAAA,FEFGN$_{131-135}$FAAAA*) pPMB21 (sc *TRP1 SUI1*) | This study |
| PMY88 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF11* pAS23 (sc *LEU2 TIF11-FGFESDE$_{121-127}$AAAAAAA,FEFGN$_{131-135}$FAAAA*) pPMB22 (sc *TRP1 SUI1-opt*) | This study |
| PMY89 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF5* p4119 (sc *TRP1 TIF5-FL*) pJCB101 (sc *LEU2 SUI1*) | This study |
| PMY90 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF5* p4119 (sc *TRP1 TIF5-FL*) pPMB10 (sc *LEU2 SUI1-opt*) | This study |
| PMY91 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF5* YCplac22 (sc *TRP1*) pJCB101 (sc *LEU2 SUI1*) | This study |
| PMY92 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF5* YCplac22 (sc *TRP1*) pPMB10 (sc *LEU2 SUI1-opt*) | This study |
| PMY93 | *MAT***a** *ura3-52 leu2-3 leu2-112 trp1*Δ*-63 his4-301*(*ACG*) *sui1*Δ*::hisG kanMX6:P$_{GAL1}$-TIF5* p4281 (sc *TRP1 TIF5-G31R*) pPMB10 (sc *LEU2 SUI1-opt*) | This study |

[a] hc, high copy number; sc, single copy.

*PYK1* ratios), they evoked ~3-fold greater increases in expression of eIF1 protein versus *SUI1* mRNA (Fig. 2, *SUI1* alleles, protein/mRNA ratios, WT versus *K60E,L96P*), thus confirming that they increase the translational efficiency of *SUI1* mRNA. Introducing the optimal context into WT *SUI1* also produced a moderate increase (~50%) in the level of *SUI1* mRNA, but the increase in eIF1 protein (2.7-fold) exceeded the increase in *SUI1* mRNA (1.5) by a factor of 1.8 (Fig. 2,

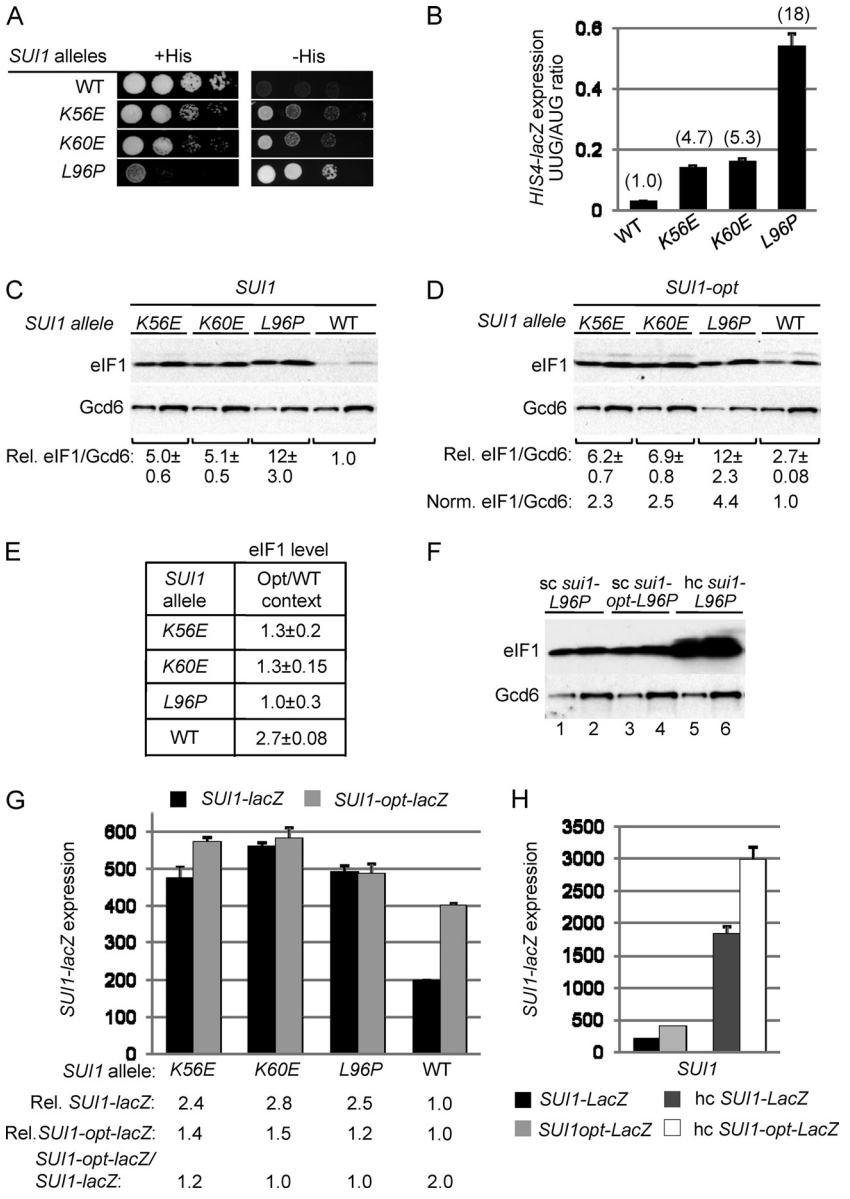
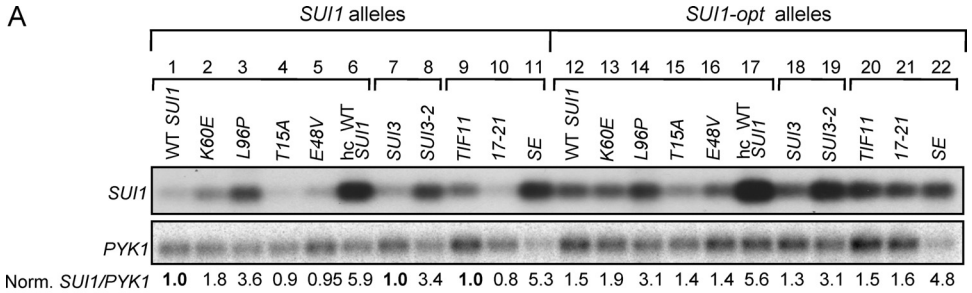



FIG. 1. *SUI1* Sui$^-$ mutations increase *SUI1* expression by suppressing poor AUG context. (A) Slg$^-$ and His$^+$/Sui$^-$ phenotypes of derivatives of *sui1*Δ *his4-301* strain JCY03 containing the indicated *SUI1* alleles on sc plasmids determined by spotting serial 10-fold dilutions on synthetic complete medium lacking leucine (Leu) supplemented with 0.3 mM histidine (+His) or 0.003 mM His (−His) and incubating for 3 days (+His) or 7 days (−His) at 30°C. (B) Strains from panel A also harboring *HIS4-lacZ* reporter plasmids with an AUG (p367) or UUG (p391) start codon were cultured in synthetic dextrose minimal medium (SD) supplemented with His and tryptophan (Trp) at 30°C to an $A_{600}$ of ~1.0, and β-galactosidase activities (nmol of *o*-nitrophenyl β-D-galactopyranoside cleaved per min per mg) were measured in WCEs. The ratio of expression of the UUG versus AUG reporter was calculated for replicate experiments, and the means and standard errors of the mean (SEM [error bar]) were plotted. Numbers in parentheses are the means normalized to the WT value. (C to E) *SUI1* Sui$^-$ mutations elevate eIF1 expression dependent on the *SUI1* AUG context. Derivatives of *sui1*Δ strain JCY03 containing the indicated *SUI1* alleles (C) or *SUI1-opt* alleles (D) were cultured in SD supplemented with His, Trp, and uracil (Ura) at 30°C to an $A_{600}$ of ~1.0, and WCEs were subjected to Western analysis using antibodies against eIF1/Sui1 or eIF2Bε/Gcd6 (analyzed as a loading control). Two different amounts of each extract differing by a factor of 2 were loaded in successive lanes. Signal intensities were quantified from replicate experiments, and mean eIF1/Gcd6 ratios were normalized to that obtained for WT *SUI1* to yield the relative (Rel.) eIF1/Gcd6 values listed below the blots. In panel D, the Rel. eIF1/Gcd6 ratios were normalized to that obtained for WT *SUI1-opt* to yield the Norm. eIF1/Gcd6 values. In panel E, the ratios of mean eIF1/Gcd6 values for *SUI1* versus *SUI1-opt* alleles were calculated for each strain. (F) Western analysis of JCY03 derivatives containing sc or hc plasmids with *sui1-L96P* (lanes 1 and 2 and lanes 5 and 6, respectively) or sc *sui1-opt-L96P* (lanes 3 and 4), conducted as in panels C and D. (G) Derivatives of JCY03 harboring the indicated sc *SUI1* alleles and sc plasmids with *SUI1-lacZ* (pPMB24) or *SUI1-opt-lacZ* (pPMB25) were cultured and assayed for β-galactosidase activities as in panel B. Mean *SUI1-lacZ* or *SUI1-opt-lacZ* expression levels determined from replicate measurements were normalized to those for WT to yield the relative (Rel.) expression values listed below the histogram, and the ratio of mean *SUI1-lacZ* versus *SUI1-opt-lacZ* expression is given on the bottom line. (H) Transformants of strain PMY30 harboring sc plasmids with *SUI1-lacZ* (pPMB24) or *SUI1-opt-lacZ* (pPMB25) and hc plasmids with *SUI1-lacZ* (pPMB26) or *SUI1-opt-lacZ* (pPMB27) reporters were cultured and assayed for β-galactosidase activities as in panel G.

A




B

| SUI1 alleles | | | | SUI1-opt alleles | | | | |
|---|---|---|---|---|---|---|---|---|
| (1) Genotype | (2) eIF1/Gcd6 Protein | (3) *SUI1*/*PYK1* mRNA | (4) Protein/ mRNA | (5) Genotype | (6) eIF1/Gcd6 Protein | (7) *SUI1*/*PYK1* mRNA | (8) Protein/ mRNA | (9) Norm. Protein/ mRNA |
| 1. WT | 1.0 | 1.0 | 1.0 | 12. WT | 2.7 | 1.5 | 1.8 | 1.0 |
| 2. *K60E* | 5.1 | 1.8 | 2.8 | 13. *K60E* | 6.9 | 1.9 | 3.6 | 2.0 |
| 3. *L96P* | 12 | 3.6 | 3.3 | 14. *L96P* | 12 | 3.1 | 3.9 | 2.2 |
| 4. *T15A* | 0.1 | 0.9 | 0.1 | 15.*T15A* | 0.8 | 1.4 | 0.6 | 0.3 |
| 5. *E48V* | 0.35 | 0.95 | 0.4 | 16. *E48V* | 1.6 | 1.4 | 1.1 | 0.6 |
| 6. hc WT | 7.0 | 5.9 | 1.2 | 17. hc WT | 16.3 | 5.6 | 2.9 | 1.6 |
| 7. *SUI3* | 1.0 | 1.0 | 1.0 | 18. *SUI3* | 2.9 | 1.3 | 2.2 | 1.0 |
| 8. *SUI3-2* | 8.7 | 3.4 | 2.6 | 19. *SUI3-2* | 6.7 | 3.1 | 2.2 | 1.0 |
| 9. *TIF11* | 1.0 | 1.0 | 1.0 | 20. *TIF11* | 2.9 | 1.5 | 1.9 | 1.0 |
| 10. *17-21* | 0.4 | 0.8 | 0.5 | 21. *17-21* | 3.3 | 1.6 | 2.1 | 1.1 |
| 11. *SE* | 7.8 | 5.3 | 1.5 | 22. *SE* | 9.9 | 4.8 | 2.1 | 1.1 |

FIG. 2. Comparison of effects of Sui$^-$ and Ssu$^-$ mutations in eIF1, eIF2β, and eIF1A on eIF1 protein and *SUI1* mRNA levels. (A) Northern analysis of *SUI1* mRNA in selected mutants. Lanes 1 to 6 and lanes 12 to 17 show results for strains described in Fig. 1C and D and in Fig. 5A, B, and F containing the indicated *SUI1*$^+$ alleles (lanes 1 to 6) or *SUI1-opt* alleles (lanes 12 to 17) cultured as in Fig. 1C. Lanes 7 to 11 and lanes 18 to 22 show the results for strains described in Fig. 6A and C containing *SUI1*$^+$ (lanes 7 to 11) or *SUI1-opt* (lanes 18 to 22) cultured as described in Fig. 6A. Total RNA was subjected to Northern analysis of *SUI1* and *PYK1* mRNAs, the hybridization signals were quantified with a Phosphorimager, and ratios of *SUI1* to *PYK1* mRNA were calculated and normalized to the ratio obtained for the corresponding strain, harboring WT *SUI1*, *SUI3*, and *TIF11*. The resulting "Norm. *SUI1*/*PYK1*" values are listed below the blot and also in columns 3 and 7 of panel B. The WT *SUI1* reference strain is shown in lane 1 for the mutants examined in lanes 1 to 6 and lanes 12 to 17. The WT *SUI1 SUI3* reference strain is shown in lane 7 for the mutants examined in lanes 7 and 8 and lanes 18 and 19. The WT *SUI1 TIF11* reference strain is shown in lane 9 for the mutants examined in lanes 9 to 11 and lanes 20 to 22. (B) Comparison of eIF1 protein and *SUI1* mRNA levels. For the strains analyzed in panel A, the appropriate "Rel. eIF1/Gcd6" values taken from Fig. 1C and D, from Fig. 5A, B, and F, and from Fig. 6A and C are listed in columns 2 and 6 (eIF1/Gcd6 Protein) for *SUI1* and *SUI1-opt* alleles, respectively; the "Norm. *SUI1*/*PYK1*" values from panel A are listed in columns 3 and 7 (*SUI1*/*PYK1* mRNA); the ratios of values in columns 2 and 3 are listed in column 4 (Protein/mRNA); the ratios of values in columns 6 and 7 are listed in column 8 (Protein/mRNA); and the values in column 8 normalized to the cognate WT ratios in rows 12, 18, or 20 are listed in column 9 (Norm. Protein/mRNA).

rows 1 and 12, protein/mRNA values), again confirming an increase in translational efficiency. After normalizing the eIF1 protein level for the corresponding *SUI1* mRNA level, it can be seen that *K60E* and *L96P* produce smaller increases in this ratio for the *SUI1-opt* allele compared to WT *SUI1* (Fig. 2, cf. rows 2 and 3, protein/mRNA, versus rows 13 and 14, Norm. Protein/mRNA), supporting the conclusion that the optimum context dampens the stimulatory effects of these Sui$^-$ mutations on the translational efficiency of *SUI1* mRNA.

In these and other experiments below, we consistently observed that mutations in the *SUI1* AUG context, or in various initiation factors, that alter expression of eIF1 protein also change *SUI1* mRNA abundance in the same direction, but to a significantly smaller extent. In fact, it is well established that translation efficiency is a major determinant of mRNA stability in yeast, since mutations in various initiation factors or the AUG context of specific mRNAs will decrease mRNA stability in proportion to their deleterious effects on translation initiation (25, 43). Hence, the simplest interpretation of the changes in *SUI1* mRNA abundance produced by mutations in initiation factors or AUG context is that they are the indirect consequences of changes in translational efficiency. As such, the eIF1 protein/*SUI1* mRNA ratios calculated in Fig. 2 likely underestimate the differences in translational efficiency conferred by these mutations.

***SUI1* Ssu$^-$ mutations reduce *SUI1* expression by exacerbating poor context.** We also screened our mutagenized *SUI1* plasmids for eIF1/Sui1 substitutions that would suppress the Sui$^-$ phenotypes of mutations in other eIFs, since this phenotype was not previously described for eIF1 mutants. Ssu$^-$ sub-

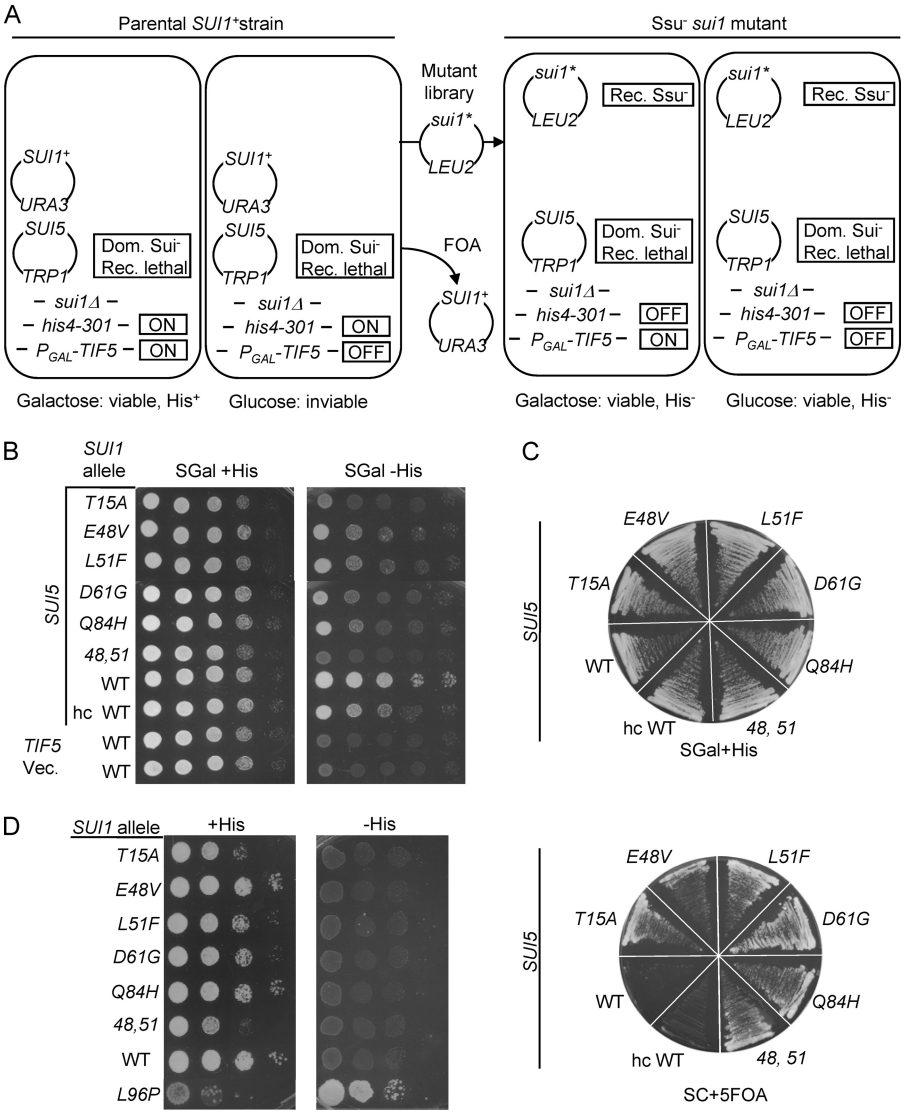



FIG. 3. Isolation of Ssu$^-$ substitutions in eIF1. (A) Summary of genetic selection used to isolate Ssu$^-$ alleles of *SUI1* as suppressors of the recessive lethality of *SUI5.* The relevant genotype, the expression levels of *his4-301* and $P_{GAL}$-*TIF5* (ON or OFF), and the growth phenotypes on medium containing galactose or glucose as carbon source are indicated for the parental strain (two rectangles on the left) and for Ssu$^-$ *sui1* mutants (two rectangles on the right). See the text for further details. (B) Ssu$^-$ phenotypes of derivatives of a *sui1*Δ *his4-301* $P_{GAL}$-*TIF5* strain with episomal *SUI5* (PMY04) harboring the indicated *SUI1* alleles. Tenfold serial dilutions of these strains plus strains PMY89 and PMY91 containing episomal *TIF5* (p4119) or empty vector, versus *SUI5* (last two rows) were spotted on SGal+His and SGal-His (containing 0.003 mM His). (C) In the top panel, the same strains as in panel B were streaked on SC containing 2% galactose, 1% raffinose as a carbon source, lacking Leu and tryptophan (Trp), and supplemented with 0.3 mM His (SGal+His). In the bottom panel, strains were streaked on SC lacking Leu and Trp and supplemented with 5-FOA (SC + 5-FOA). (D) Phenotypes of *SUI1* Ssu$^-$ mutations in the absence of *SUI5*. Derivatives of *sui1*Δ *his4-301* strain JCY03 containing the indicated *SUI1* alleles were analyzed as in Fig. 1A.

stitutions were of interest because they should increase the accuracy of initiation and reinstate the requirement for an AUG start codon for efficient initiation in Sui$^-$ mutants. It was of interest to determine whether such Ssu$^-$ substitutions would also impose a stronger requirement for optimal AUG context for efficient initiation.

Ssu$^-$ alleles of *SUI1* were isolated by their ability to suppress the recessive lethality of the *SUI5* mutation in eIF5 (Tif5 in yeast), the GTPase activating protein for eIF2. As diagrammed in Fig. 3A, we used a *sui1*Δ *his4-301* strain containing plasmid-borne copies of *SUI1*$^+$ and *SUI5*, plus a chromosomal *TIF5* allele under the *GAL1* promoter ($P_{GAL}$-*TIF5*) that expresses WT eIF5 only on galactose medium. This strain can grow on galactose medium lacking histidine owing to the dominant Sui$^-$ phenotype of *SUI5* and the complementation of its recessive lethality by $P_{GAL1}$-*TIF5*; however, the strain cannot grow on glucose medium because $P_{GAL1}$-*TIF5* is repressed, and *SUI5* is the only source of eIF5 under these conditions (Fig. 3A, left). Hence, we selected plasmids from the mutant library that, following eviction of the *SUI1*$^+$ plasmid on 5-FOA medium, rescued the ability to grow on glucose medium (suppressing *SUI5* lethality) and eliminated the His$^+$ phenotype on

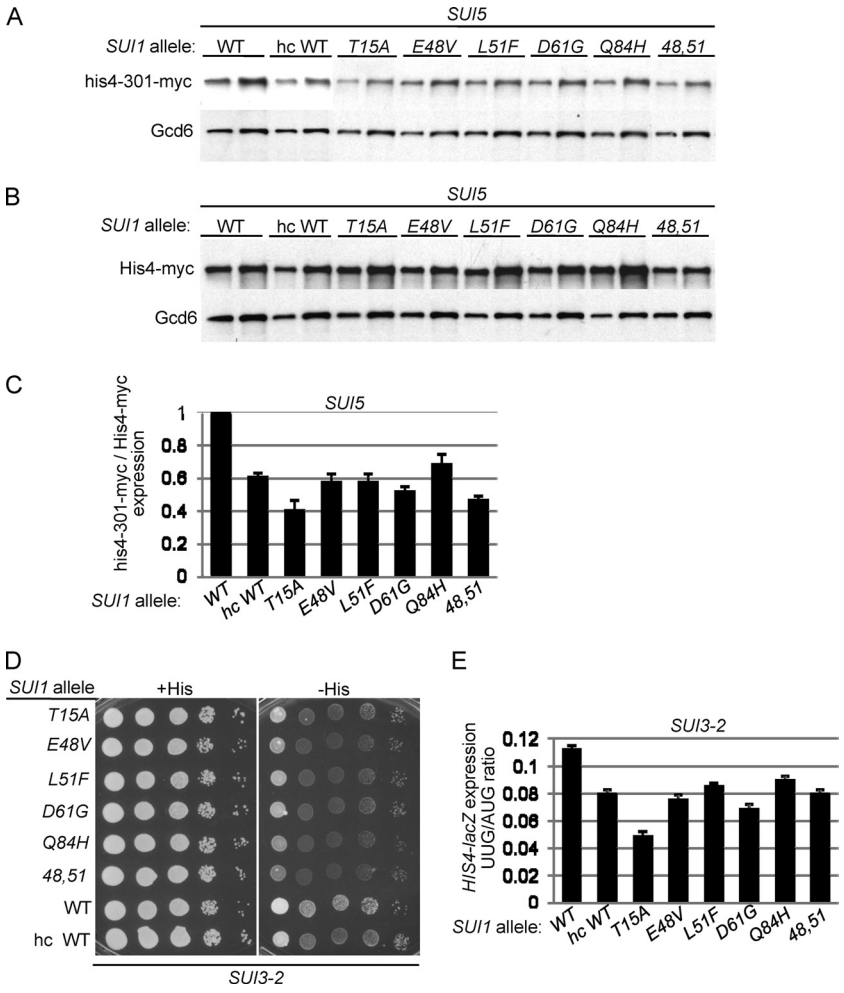



FIG. 4. *SUI1* Ssu$^-$ mutations reduce the *HIS4* UUG/AUG initiation ratio in *SUI5* and *SUI3-2* cells. (A to C) Derivatives of *sui1*Δ *his4-301-myc SUI5* (A) and *sui1*Δ *HIS4-myc SUI5* (B) strains JCY04 and PMY16, respectively, harboring the indicated *SUI1* alleles were cultured as in Fig. 1C, and WCEs were subjected to Western analysis with antibodies against myc epitope or Gcd6. Two different amounts of each extract differing by a factor of 2 were loaded in successive lanes. (C) Western signals from panels A and B were quantified, and the mean ratios of his4-301-myc to His4-myc (each normalized to Gcd6) are plotted with the SEM as error bars. (D) Derivatives of JCY03 containing episomal *SUI3-2* (p4280/YCpSUI3-S264Y-W) and harboring the indicated *SUI1* alleles were analyzed for Slg$^-$ and His$^+$/Sui$^-$ phenotypes by spotting serial 10-fold dilutions on SC lacking Leu and Trp and supplemented with either 0.3 mM His (+His) or 0.003 mM His (−His). (E) Transformants of the *SUI3-2* strains from panel D containing the AUG or UUG *HIS4-lacZ* reporters were analyzed as in Fig. 1B.

galactose medium (suppressing the *SUI5* Sui$^-$ phenotype) (Fig. 3A, right). We focused on five such *sui1* alleles that introduce single amino acid substitutions into eIF1—*T15A*, *E48V*, *L51F*, *D61G*, and *Q84H*—and by site-directed mutagenesis generated a sixth allele that combines two of the mutations, *E48V* and *L51F* (abbreviated as "*48,51*"). The results in Fig. 3B (right) document that these *sui1* alleles suppress the His$^+$/Sui$^-$ phenotype of *SUI5*, while those in Fig. 3C (bottom) demonstrate cosuppression of *SUI5* lethality. In the absence of *SUI5*, these *sui1* alleles produce slight to moderate slow-growth phenotypes and, as expected, do not confer Sui$^-$ phenotypes on their own (Fig. 3D).

We verified the Ssu$^-$ phenotypes of these mutants by Western analysis of the myc-tagged version of the *his4-301* product expressed in these strains. Expression of WT His4-myc was measured in parallel in isogenic strains harboring *HIS4-myc* (with AUG start codon) versus *his4-301-myc* (Fig. 4A and B). Quantification of the Western data revealed that, compared to the *SUI1*$^+$ strain, all of the mutants display significantly reduced levels of his4-301-myc versus His4-myc (Fig. 4C), indicating a decreased UUG to AUG initiation ratio for *HIS4* mRNA in the manner expected for Ssu$^-$ alleles. Note also that the mutants resemble the *SUI1*$^+$ strain that overexpresses WT eIF1 from an hc *SUI1*$^+$ plasmid (hc WT), which is known to confer an Ssu$^-$ phenotype (Fig. 4C) (48).

To confirm these last findings, we examined whether the *sui1* Ssu$^-$ mutations also suppress the Sui$^-$ phenotype of the dominant eIF2β Sui$^-$ allele *SUI3-2* (21). Indeed, all of the *sui1* alleles resemble hc *SUI1*$^+$ in suppressing the His$^+$ phenotype of *SUI3-2* (Fig. 4D) and in lowering the UUG/AUG initiation ratio for the matched *HIS4-lacZ* fusions in the *SUI3-2* strain (Fig. 4E). The fact that they mitigate the Sui$^-$ phenotype of *SUI3-2* confirms that all six *sui1* alleles have Ssu$^-$ phenotypes.

Remarkably, all of the Ssu$^-$ *sui1* alleles exhibit levels of eIF1

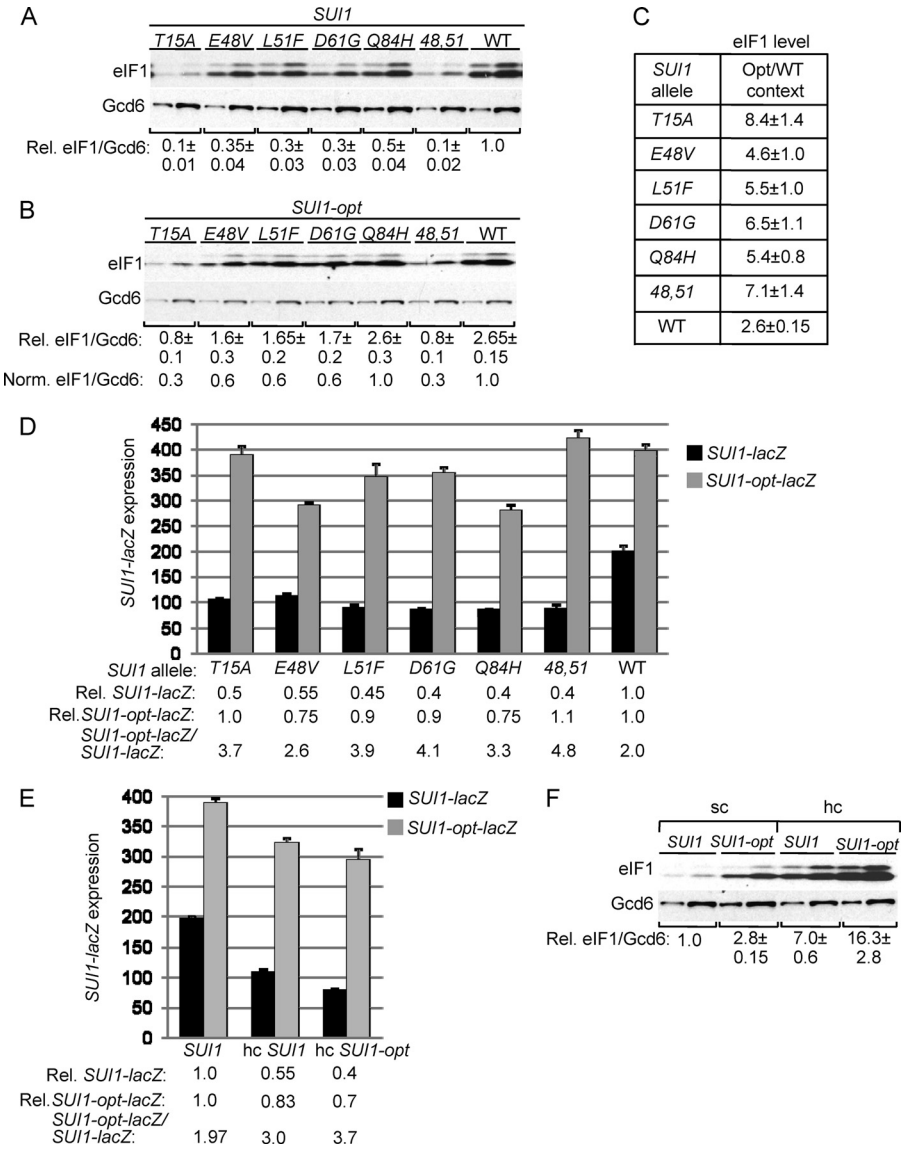



FIG. 5. *SUI1* Ssu$^-$ mutations reduce *SUI1* expression by exacerbating poor context. (A to C) *SUI1* Ssu$^-$ mutations reduce eIF1 expression dependent on *SUI1*$^+$ AUG context. Derivatives of JCY03 containing the indicated *SUI1* alleles (A) or *SUI1-opt* alleles (B) were subjected to Western analysis as in Fig. 1C. Two different amounts of each extract differing by a factor of 2 were loaded in successive lanes. (C) Ratios of mean eIF1/Gcd6 values for *SUI1* versus *SUI1-opt* alleles were calculated as in Fig. 1E. (D) Derivatives of JCY03 harboring the indicated *SUI1* alleles and the *SUI1-lacZ* or *SUI1-opt-lacZ* reporter were analyzed for β-galactosidase activities as in Fig. 1G. (E) *SUI1-lacZ* and *SUI1-opt-lacZ* expression levels were determined in derivatives of JCY03 containing sc *SUI1*, hc *SUI*, or hc *SUI1*-opt, as indicated, as in Fig. 1G. (F) Western analysis of eIF1 expression in JCY03 derivatives containing the indicated sc or hc *SUI1* alleles, conducted as in Fig. 1C.

expression lower than that of the WT, with the *T15A* and *48,51* mutants decreasing expression the most, by a factor of ~10 (Fig. 5A). These results suggest that the Ssu$^-$ substitutions exacerbate the effect of poor context at the *SUI1* start codon. Supporting this interpretation, the reduction in eIF1 expression conferred by these *sui1* mutations was substantially alleviated, or completely eliminated, by the presence of the optimal AUG context in the cognate *SUI1-opt* alleles (Fig. 5B). For example, whereas *T15A* and *48,51* reduced expression of WT *SUI1* by ~10-fold (Fig. 5A), they reduced expression of *SUI1-opt* by only ~3-fold (Fig. 5B). Note also that whereas optimal context increases eIF1 expression from *SUI1*$^+$ by a factor of 2.6, it provokes substantially larger increases, between 4.6- and 8.4-fold, in eIF1 expression from the Ssu$^-$ alleles (Fig. 5C), as expected if the latter exacerbate the effect of poor context at the *SUI1* AUG codon. The fact that *T15A* and *48*,*51* produce considerably lower than WT levels of eIF1 even in the presence of the optimal context (Fig. 5B) might indicate that these substitutions destabilize eIF1 protein in addition to decreasing AUG recognition. Alternatively, another unknown feature of *SUI1* mRNA might be suboptimal, and these Ssu$^-$ substitutions would discriminate against this hypothetical anti-determinant in addition to poor AUG context. For example, it was shown that coding sequences function coordinately with the

AUG context to stimulate the efficiency of translation and stability of *PGK1* mRNA in yeast (25).

To confirm the conclusions reached from Western analysis, we also examined the effects of the Ssu$^-$ mutations in *trans* on *SUI1-lacZ* expression. All of the mutations decrease expression of the WT fusion by factors of 2 to 2.5 (Fig. 5D, Rel. *SUI1-lacZ*) but, importantly, have considerably less effect on expression of the *SUI1-opt-lacZ* fusion (Fig. 5D, Rel. *SUI1-opt-lacZ*). Furthermore, whereas introducing the optimal context increases fusion gene expression by a factor of 2.0 in WT cells, it produces larger increases, between 2.6- and 4.8-fold, in the Ssu$^-$ mutants (Fig. 5D, *SUI1-opt-lacZ*/*SUI1-lacZ*). These findings support the conclusion that eIF1 Ssu$^-$ substitutions reduce *SUI1* expression by exacerbating its poor AUG context.

Consistent with their deleterious effects on eIF1 expression, Northern analysis revealed that the *T15A* and *E48V* mutations also reduced the level of *SUI1* mRNA. However, since the magnitude of the decrease in mRNA is less than the reduction in eIF1 protein levels evoked by these mutations, they confer protein/mRNA ratios of less than unity (Fig. 2, rows 4 and 5, Protein/mRNA), supporting the conclusion that the mutations reduce *SUI1* translational efficiency. Moreover, the eIF1 protein/*SUI1* mRNA ratios are reduced by the *T15A* and *E48V* mutations to a greater extent for *SUI1* versus *SUI1-opt* alleles (Fig. 2, rows 4 and 5 [Protein/mRNA] versus rows 15 and 16 [Norm. Protein/mRNA]), supporting the idea that they reduce *SUI1* translational efficiency by intensifying the negative effect of its native, poor AUG context.

**eIF1 moderately autoregulates translation by exacerbating the effects of poor AUG context at *SUI1*.** As noted above, overexpressing WT eIF1 confers an Ssu$^-$ phenotype (41, 48). Consistent with this, hc *SUI1*$^+$ phenocopies the *sui1* Ssu$^-$ mutations and reduces expression of the WT *SUI1-lacZ* fusion by a factor of 2 but diminishes expression of *SUI1-opt-lacZ* by only ~20% (Fig. 5E, *SUI1* versus hc *SUI1*). The repression of *SUI1-lacZ* expression occurs in response to an ~7-fold increase in eIF1 expression from hc *SUI1* versus sc *SUI1* (Fig. 5F). We noted that ~2-fold greater eIF1 expression (16-fold above WT) was achieved with the hc version of *SUI1-opt* versus hc *SUI1* (Fig. 5F), which is consistent with the 2-fold greater translational efficiency of *SUI1-opt* versus *SUI1* determined above (Fig. 1C, D, and G and Fig. 2). Consistent with this, hc *SUI1-opt* conferred more extensive repression of *SUI1-lacZ* expression than did hc *SUI1*, plus an increased stimulatory effect of introducing the optimal context into the *lacZ* fusion (Fig. 5E, cf. *SUI1-opt-lacZ*/*SUI1-lacZ* ratios). These findings suggest that eIF1 negatively autoregulates its synthesis by exacerbating the deleterious effect of the poor AUG context in *SUI1* mRNA when the eIF1 level increases in the cell. On the other hand, the autoregulation is not efficient enough to prevent considerable eIF1 overexpression and the attendant Ssu$^-$ phenotype in response to elevated *SUI1* dosage. As shown below, this might reflect the fact that the native *SUI1* context is not fully suboptimal.

**Sui$^-$ mutations in eIF2β and eIF1A also suppress the poor AUG context at *SUI1*.** The results presented above indicate that Sui$^-$ substitutions in eIF1 that reduce its discrimination against UUG codons in *HIS4* mRNA likewise reduce the deleterious effect of poor AUG context in *SUI1* mRNA. We hypothesized that other factors involved in rejecting near-cognate start codons, including eIF2β, eIF1A, and eIF5, also function in rejecting poor AUG context. Accordingly, we sought to determine whether Sui$^-$ mutations in these factors provoke increased expression of *SUI1* dependent on its poor context. To examine the effect of the Sui$^-$ eIF2β mutation *SUI3-2*, we generated strains with chromosomal *SUI3*$^+$ under the *GAL1* promoter and harboring plasmid-borne *SUI3-2* or *SUI3*, which express only the plasmid-encoded S264Y mutant, or WT, eIF2β on glucose medium.

Remarkably, the *SUI3-2* transformant displayed a strong, 8.7-fold increase in eIF1 expression from WT *SUI1* but only a 2.3-fold increase in eIF1 expression from *SUI1-opt* (Fig. 6A). In addition, optimizing the context produced no increase in eIF1 expression in the *SUI3-2* cells (Fig. 6D). Analysis of mRNA levels revealed that *SUI3-2* conferred a 2.6-fold increase in the eIF1 protein/*SUI1* mRNA ratio for *SUI1*$^+$ but had almost no effect on this ratio for *SUI1-opt* (Fig. 2, row 8, Protein/mRNA versus row 19, Norm. Protein/mRNA), confirming a significant increase in translational efficiency exclusively for the suboptimal, native AUG context. The same conclusion emerged from analysis of the *SUI1-lacZ* fusions, as *SUI3-2* provoked 4.6-fold higher expression of *SUI1-lacZ*, but only 1.9-fold higher expression of *SUI1-opt-lacZ*, relative to the levels observed in *SUI3*$^+$ transformants (Fig. 6B). Moreover, introducing the optimal context produced only a slight (~20%) increase in fusion gene expression in *SUI3-2* cells, compared to the 3-fold higher expression seen in *SUI3* cells (Fig. 6B). These findings indicate that *SUI3-2* strongly suppresses the effect of poor context at *SUI1*. Thus, eIF2β (presumably in the context of the eIF2 holoprotein) discriminates against poor AUG context in addition to its known function in blocking non-AUG initiation.

To evaluate whether a Sui$^-$ substitution affecting eIF1A also overcomes poor context, we took the approach described above and examined transformants harboring the plasmid-borne eIF1A Sui$^-$ allele *tif11-SE$_1$*,SE$_2$*+F131* (abbreviated *SE* below), or WT *TIF11*, in a strain where chromosomal *TIF11*$^+$ is glucose repressible. The *SE* mutation, which impairs both scanning enhancer elements (SE$_1$ and SE$_2$) in the eIF1A C-terminal tail, greatly elevates the UUG/AUG ratio for the *HIS4-lacZ* fusion (41). We also examined the Ssu$^-$ mutation *tif11-17-21*, which introduces substitutions into the scanning inhibitory (SI) element in the N-terminal tail of eIF1A and lowers the UUG/AUG ratio in cells harboring *SUI3-2* (14, 41).

Consistent with our findings on other Sui$^-$ mutations, the eIF1A *SE* mutation provoked an ~8-fold increase in eIF1 expression from WT *SUI1* compared to an ~3-fold increase for the *SUI1-opt* allele (Fig. 6C), and introducing the optimal context increased eIF1 expression by only 1.3-fold in *SE* mutant cells compared to 2.9-fold in *TIF11*$^+$ cells (Fig. 6D). We observed a more substantial increase in *SUI1* mRNA in the *SE* mutant than in other Sui$^-$ mutants, so that the eIF1 protein/*SUI1* mRNA ratio was only 50% higher in *SE* versus *TIF11*$^+$ cells (Fig. 2, row 11, Protein/mRNA). However, the *SE* mutation produced a larger increase in expression of *SUI1-lacZ* versus *SUI1-opt-lacZ*, of 3.1- versus 1.9-fold, respectively, and introducing the optimal context increased *SUI1-lacZ* expression only slightly (by 15%) in the *SE* mutant compared to the 2-fold increase observed in WT cells (Fig. 6E). Taken together, the results indicate that the eIF1A *SE* mutation mitigates the

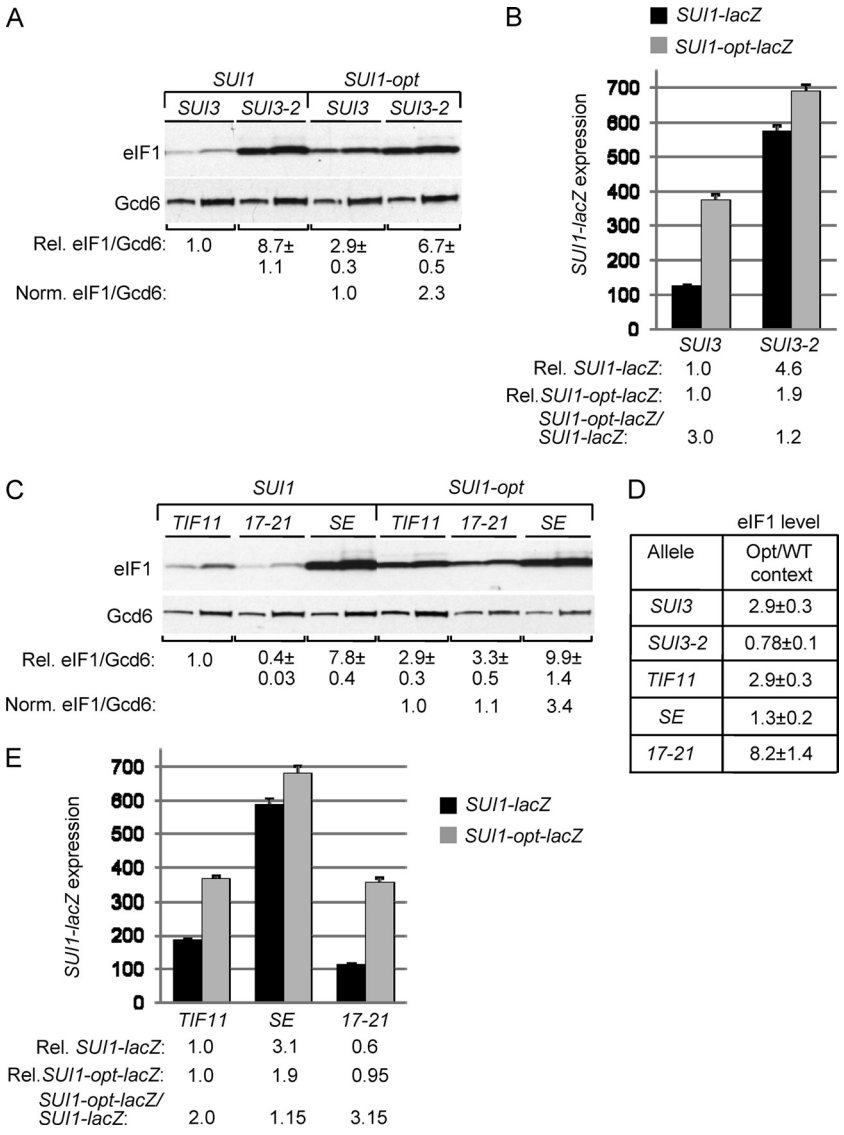



FIG. 6. Sui$^-$ mutations in eIF2β and eIF1A suppress the poor AUG context at *SUI1*. (A) Derivatives of *sui1*Δ $P_{GAL}$-*SUI3* strain PMY02 containing plasmid-borne *SUI3* (p4450) or *SUI3-2* (p4280) and either sc *SUI1*$^+$ or sc *SUI1-opt* were cultured continuously in SD supplemented with His and Ura (with repression of chromosomal $P_{GAL}$-*SUI3*) and subjected to Western analysis of eIF1 expression, as in Fig. 1C. Two different amounts of each extract differing by a factor of 2 were loaded in successive lanes. (B) Analysis of *SUI1-lacZ* and *SUI1-opt-lacZ* expression in the *SUI1*$^+$ strains from panel A conducted as in Fig. 1G. (C) Western analysis of eIF1 in derivatives of *sui1*Δ $P_{GAL}$-*TIF11* strain PMY03 containing plasmid-borne *TIF11*$^+$ (pDSO9), *tif11-SE1*,SE2*+F131*(pAS23), or *tif11-17–21* (p4552), and either sc *SUI1*$^+$ or sc *SUI1-opt*, conducted as in panel A. (D) Ratios of mean eIF1/Gcd6 values for *SUI1* versus *SUI1-opt* alleles calculated for strains analyzed in panels A and C. (E) Analysis of *SUI1-lacZ* and *SUI1-opt-lacZ* expression in transformants of the strains from the *SUI1*$^+$ strains from panel C, as in Fig. 1G.

deleterious effect of poor AUG context, as described above for Sui$^-$ mutations in eIF1 and eIF2β.

The eIF1A Ssu$^-$ mutation *tif11-17-21* reduced the level of eIF1 expressed from *SUI1* but not from *sui1-opt* (Fig. 6C) and increased the stimulatory effect of introducing optimal AUG context on eIF1 expression from ~3-fold (in WT) to ~8-fold (Fig. 6D). The *17-21* mutation also produced an obvious reduction in the eIF1 protein/*SUI1* mRNA ratio for *SUI1*, but not *SUI1-opt* (Fig. 2, row 10, Protein/mRNA, versus row 21, Norm. Protein/mRNA), and it decreased expression of the *SUI1-lacZ* fusion but not that of *SUI1-opt-lacZ* (Fig. 6E). These results indicate that, like the Ssu$^-$ mutations in eIF1 discussed above, the eIF1A Ssu$^-$ mutation *17-21* confers a significant decrease in *SUI1* translation that depends on its poor AUG context.

Finally, we examined the effect of the eIF5 Sui$^-$ substitution (G31R) encoded by the *SUI5* allele of *TIF5*. As noted above, *SUI5* is lethal as the only source of eIF5 but produces a dominant Sui$^-$ phenotype and a large increase in the UUG/AUG ratio in cells coexpressing WT eIF5 (21). Hence, we first examined the effect of *SUI5* on eIF1 and *SUI1-lacZ* expression in a strain containing chromosomal $P_{GAL}$-*TIF5*, and cultured cells on galactose to allow coexpression of WT eIF5 and attendant cell growth. Under these conditions, *SUI5* produced

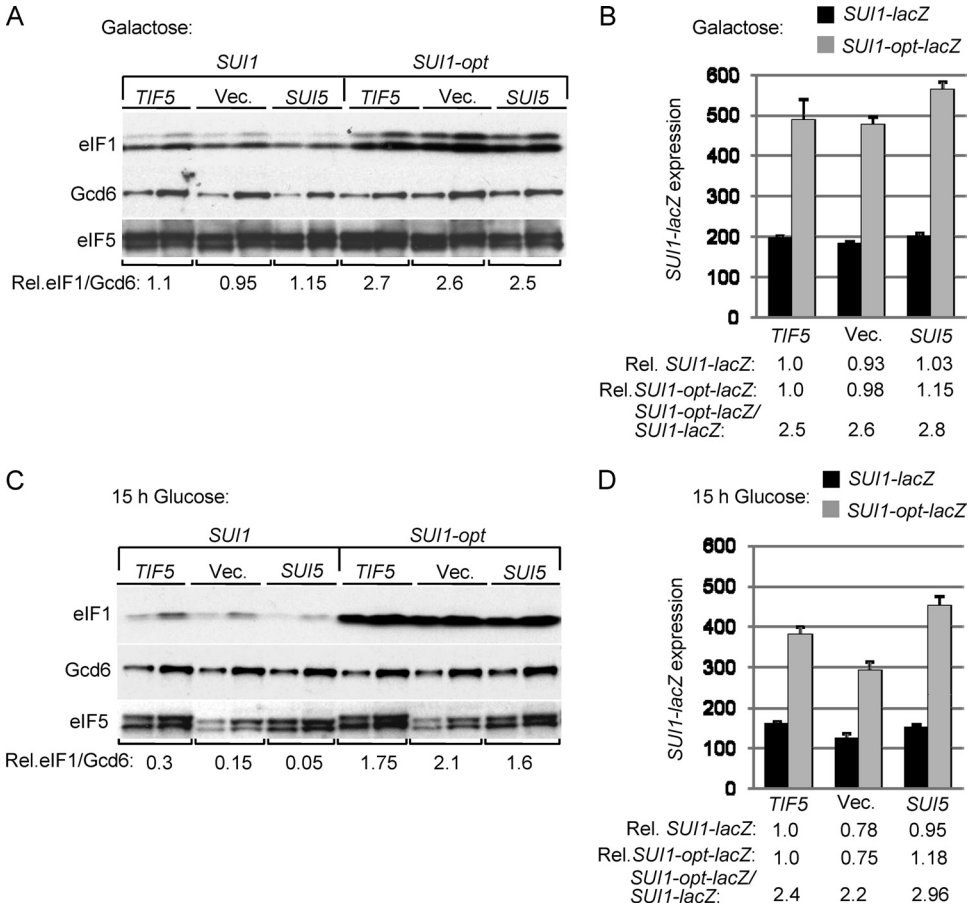



FIG. 7. The eIF5 Sui$^-$ mutation *SUI5* does not suppress poor AUG context at *SUI1*. (A and C). Western analysis of eIF1 expression in derivatives of *sui1*Δ $P_{GAL}$-*TIF5* strain PMY01 containing plasmid-borne *TIF5-FL* (p4119), empty vector (YCplac22) or *SUI5* (p4281) and either sc *SUI1*$^+$ or sc *SUI1-opt*, conducted as in Fig. 1C except that strains were cultured in synthetic minimal medium with 2% galactose as carbon source and histidine and uracil supplements (SGal+HU) (A) and then shifted to SD+HU for 15 h (C). Two different amounts of each extract differing by a factor of 2 were loaded in successive lanes. (B and D) Analysis of *SUI1-lacZ* and *SUI1-opt-lacZ* expression in transformants of *SUI1*$^+$ strains from panels A and C, cultured as described there.

little or no change in eIF1 expression from *SUI1*$^+$ or *SUI1-opt* (Fig. 7A, Vec. versus *SUI5* lanes), nor did it alter expression of *SUI1-lacZ* or *SUI1-opt-lacZ* (Fig. 7B). We repeated the analysis after shifting cells to glucose medium to repress eIF5 production from the chromosomal $P_{GAL}$-*TIF5* allele. Western analysis confirmed the expected reduction in total eIF5 level in cells harboring empty vector versus episomal *SUI5* or *TIF5*$^+$ (Fig. 7C, *SUI1* lanes, eIF5 blot). Under these conditions, we observed a reduction in eIF1 expression in the cells harboring episomal *SUI5* versus *TIF5*$^+$ or empty vector in the *SUI1*$^+$, but not *SUI1-opt*, strains (Fig. 7C), which could indicate that *SUI5* differs from other Sui$^-$ mutants in exacerbating rather than suppressing the effect of poor context at *SUI1*. However, this effect was not observed when expression of the *SUI1-lacZ* and *SUI1-opt-lacZ* fusions were assayed under the same conditions (Fig. 7D). Similar results were obtained in a strain containing chromosomal *TIF5*$^+$ instead of $P_{GAL}$-*TIF5* (data not shown). Thus, despite the fact that *SUI5* elevates initiation from the near-cognate UUG start codon at *HIS4* to confer a Sui$^-$ phenotype, it does not overcome the effect of poor context at *SUI1* and thereby elevate eIF1 protein expression.

**eIF1, eIF1A, and eIF2β discriminate generally against poor AUG context.** Although the context of WT *SUI1* is expected to be unfavorable, we sought to determine whether Sui$^-$ mutations in eIF1, eIF2β, and eIF1A also discriminate against a different context that deviates strongly from the yeast optimum. Since previous studies in mammalian cells identified $U_{-3}$-$U_{-2}$-$U_{-1}$-AUG as a highly unfavorable context (23), we introduced it into *SUI1-lacZ*, producing the *SUI1-*$_{UUU}$*-lacZ* construct, and examined its effect on fusion expression in different mutants. Interestingly, the $U_{-3}$-$U_{-2}$-$U_{-1}$-AUG context reduced fusion expression compared to the WT *SUI1-lacZ* construct, such that *SUI1-*$_{UUU}$*-lacZ* is expressed at a level ~3.5-fold below that of *SUI1-opt-lacZ* (Fig. 8A). Importantly, these differences in expression are nearly eliminated by Sui$^-$ mutations in eIF1 (*K60E*), eIF2β (*SUI3-2*), and eIF1A (*SE*), while the difference in expression between *SUI1-*$_{UUU}$*-lacZ* and *SUI1-opt-lacZ* is exacerbated by the Ssu$^-$ mutations affecting eIF1 (*E48V*) and eIF1A (*17-21*) (Fig. 8). These findings demonstrate that the effects of these Sui$^-$ and Ssu$^-$ mutations are not restricted to the native, poor context at *SUI1* and likely apply more generally to deviations from the optimal context.

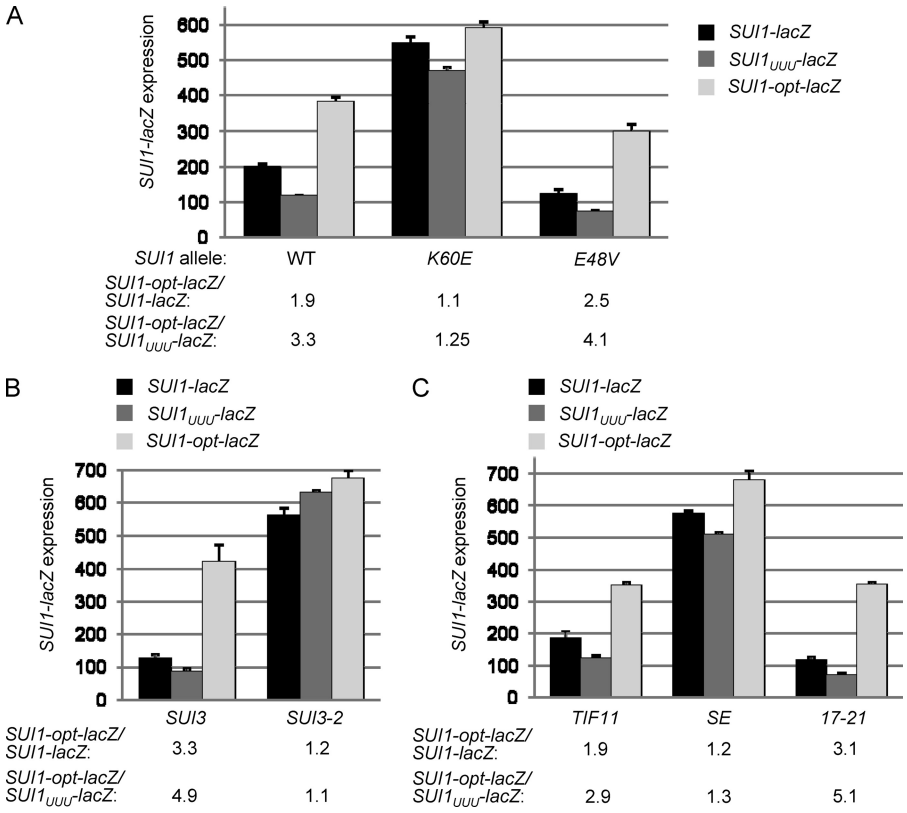



FIG. 8. Sui$^-$ and Ssu$^-$ mutations in eIF1, eIF2β and eIF1A modulate the deleterious effects of the $U_{-3}$-$U_{-2}$-$U_{-1}$-AUG context. (A to C). Analysis of expression of reporter plasmids *SUI1-lacZ* (pMB24), $SUI1_{UUU}$*-lacZ* (pMB28), and *SUI1-opt-lacZ* (pMB25) in derivatives of *sui1*Δ *his4-301* strain JCY03 containing the indicated sc *SUI1* alleles (A), derivatives of *sui1*Δ $P_{GAL}$*-SUI3* strain PMY02 containing plasmid-borne *SUI3* (p4450) or *SUI3-2* (p4280) and sc $SUI1^+$ (pJCB101) (B), and derivatives of *sui1*Δ $P_{GAL}$*-TIF11* strain PMY03 containing plasmid-borne $TIF11^+$ (pDSO9), *tif11-SE1**, *SE2*+F131* (pAS23), or *tif11-17-21* (p4552), and sc $SUI1^+$ (pJCB101) (C), as in Fig. 1G.

They also show that the native *SUI1* context is not fully suboptimal, since the $U_{-3}$-$U_{-2}$-$U_{-1}$-AUG context appears to be even less efficient.

## DISCUSSION

In this study, we have shown that eIF1/Sui1, eIF2β/Sui3, and eIF1A/Tif11 all participate in recognition of the sequence context of initiation codons *in vivo* and utilize specific residues or domains to discriminate against both poor sequence context and non-AUG start codons. As a result, alterations in these factors that increase (Sui$^-$) or decrease (Ssu$^-$) utilization of a UUG start codon at *HIS4* also increase or decrease, respectively, initiation at the $SUI1^+$ AUG codon in a manner that depends on its native, poor context. The Sui$^-$ and Ssu$^-$ mutations in eIF1, eIF2β, and eIF1A similarly suppress or exacerbate, respectively, the negative effect of the $U_{-3}$-$U_{-2}$-$U_{-1}$-AUG context, suggesting that these factors act generally to regulate context effects *in vivo*. Overexpressing WT eIF1 reduces the initiation frequency at UUG start codons in various *sui* mutants (Ssu$^-$ phenotype) and, similarly, we found that eIF1 overexpression decreases initiation at the *SUI1* AUG in the native, poor context. This enables eIF1 to negatively autoregulate its synthesis, by reducing *SUI1* mRNA translation when the cellular concentration exceeds the native, steady-state level.

Among the key observations supporting the aforementioned conclusions is our finding that novel Sui$^-$ mutations in eIF1 and the previously described Sui$^-$ mutations in eIF2β (*SUI3-2*) and eIF1A (*tif11-SE*) all confer strong increases in eIF1 protein expression from $SUI1^+$ but not from the *SUI1-opt* allele, the latter containing a perfect match to the yeast consensus context of $A_{-3}$-$A_{-2}$-$A_{-1}$-AUG (44) that we found to elevate eIF1 abundance in otherwise WT cells. Furthermore, the same effects were observed for the corresponding *SUI1-lacZ* and *SUI1-opt-lacZ* fusions, where the effects of the eIF1 mutations, as well as those in eIF2β and eIF1A, are exerted in *trans.* Conversely, our novel Ssu$^-$ substitutions in eIF1, and the previously described Ssu$^-$ mutation *17-21* of eIF1A, more strongly repressed expression of $SUI1^+$ compared to *SUI1-opt.* The fact that the optimum consensus in *SUI1-opt* masks the effects of these mutations on *SUI1* expression constitutes strong genetic evidence that these mutations modulate *SUI1* expression by suppressing (in Sui$^-$ mutants) or exacerbating (in Ssu$^-$ mutants) the deleterious effects of poor context on recognition of the *SUI1* AUG codon.

The increase or decrease in eIF1 protein expression produced by the Sui$^-$ and Ssu$^-$ mutations, respectively, were accompanied by increases or decreases, respectively, in the level of *SUI1* mRNA. It could be argued that the mutations have a direct effect on *SUI1* transcription or mRNA stability

rather than on translation initiation. This seems quite improbable, however, because the mutations affect factors or mRNA sequences with well-established functions in translation initiation, and the eIF mutations were originally selected by their ability to alter the stringency of AUG selection. Another possibility is that the observed changes in mRNA levels are elicited by the nonsense-mediated decay pathway (NMD). Owing to the poor context of the *SUI1* start codon, a proportion of scanning PICs might bypass this AUG codon and initiate 100 nt downstream at an out-of-frame five-codon ORF, triggering NMD on translation termination. Introducing the optimal context or a Sui$^-$ mutation would suppress leaky scanning of the first AUG and block the NMD response, thus increasing *SUI1* mRNA abundance, as we observed in these situations. Ssu$^-$ mutations, by contrast, would exacerbate the effect of poor context, increase leaky scanning of the first AUG codon and intensify the effect of NMD in lowering *SUI1* mRNA abundance. At odds with this possibility, however, He et al. reported that *SUI1* mRNA abundance is not altered by inactivation of NMD in different *upf* mutants (18). Thus, in view of previous findings that mRNA stability is coupled to translational efficiency in yeast (25, 43), the simplest interpretation of our findings seems to be that the changes in translational efficiency produced by the eIF mutations under study lead indirectly to alterations in mRNA degradation by a non-NMD mechanism.

Previous work led to the conclusion that eIF1 and eIF1A bind to the 40S subunit and stabilize an open conformation that is conducive to recruitment of TC and scanning, but incompatible with start codon selection. In the open conformation, the anticodon stem-loop (ASL) of Met-tRNA$_i^{Met}$ occupies the P site in a way that enables inspection of successive triplets for base-pairing with the initiator anticodon without triggering downstream events in the pathway. Furthermore, while the GTP in a fraction of the 43S complexes is hydrolyzed, release of $P_i$ from eIF2-GDP-$P_i$ is blocked in the open complex. Entry of AUG in the P site triggers a series of events that are precipitated, or facilitated, by dissociation of eIF1 from the 40S subunit. These include rearrangement to a closed, scanning-incompatible 40S conformation, in which Met-tRNA$_i^{Met}$ is fully accommodated and more tightly bound to the P site, plus release of $P_i$ from eIF2-GDP-$P_i$ to complete the GTP hydrolysis reaction.

Most Sui$^-$ mutations in eIF1 analyzed previously weaken its binding to the 40S and thereby promote rearrangement from the open to closed PIC conformation. Although the reduced 40S occupancy of a Sui$^-$ eIF1 mutant decreases the rate of TC loading, since this reaction occurs in the open 40S conformation, once TC is bound it can isomerize more readily to the fully accommodated mode of P-site binding in the absence of a perfect codon-anticodon match, e.g., at UUG codons, thus accounting for the Sui$^-$ phenotype (8, 33). We presume that the novel Sui$^-$ mutations in eIF1 described here similarly reduce its affinity for the 40S because they exhibit the hallmark of this class of eIF1 mutations, that their Sui$^-$ phenotypes are suppressed by overexpressing the mutant proteins (unpublished observations). It is thought that increasing the cellular concentration of eIF1 proteins harboring such Sui$^-$ substitutions overcomes their 40S binding defects by mass action, reversing premature eIF1 release at non-AUG codons. This is the same mechanism evoked to explain how overexpressing WT eIF1 suppresses the Sui$^-$ phenotypes of mutations in other eIFs (8, 48).

We found that the novel Ssu$^-$ mutations in eIF1 suppress the Sui$^-$ phenotypes of both *SUI3-2* and *SUI5* mutations in eIF2β and eIF5, respectively. Ssu$^-$ mutations in eIF1A with these properties have been shown to shift the equilibrium toward the open conformation (41), with increased retention of eIF1 on the 40S subunit (8), suppressing both the defect in TC loading to the open complex and the inappropriate rearrangement to the closed complex at UUG provoked by Sui$^-$ mutations in the eIF1A SE elements (41). The fact that the *17-21* Ssu$^-$ mutation in eIF1A also diminishes the Sui$^-$ and TC loading defects conferred by the *SUI3-2* mutation in eIF2β (14, 41) suggests that *SUI3-2* produces a Sui$^-$ phenotype, at least partly, by destabilizing TC binding to the open conformation (41). There is biochemical evidence that elevated GTP hydrolysis by the TC also plays a role (21), possibly shifting the equilibrium between eIF2-GTP and eIF2-GDP-$P_i$ to the right and driving $P_i$ release at non-AUG codons by mass action, or increasing the rate of $P_i$ release directly. This latter defect of *SUI3-2* could also be mitigated by the ability of the *17-21* mutation to stabilize the open conformation and retard eIF1 dissociation. Suppression of the eIF5 Sui$^-$ mutation *SUI5* by *17-21* (14) can be explained similarly, since this eIF5 substitution both destabilizes the open conformation of the PIC (29) and accelerates GTP hydrolysis at UUG codons (21). Hence, we propose that the novel Ssu$^-$ substitutions in eIF1 described here likewise impede the open-to-closed transition of the PIC, possibly by retarding eIF1 dissociation, as a means of suppressing UUG initiation in cells harboring the *SUI5* or *SUI3-2* Sui$^-$ substitutions in eIF5 or eIF2β, respectively.

Our finding that Sui$^-$ substitutions in eIF1 and eIF1A increase utilization of AUG codons in poor context in addition to enhancing initiation at UUG codons can be readily understood in the context of the model for scanning and AUG recognition described above. As suggested previously by Pestova and coworkers (37), we envision that optimal context is another feature besides the perfect AUG-anticodon duplex that stabilizes the closed conformation of the PIC, such that a poor context will impede the rearrangement from open to closed conformation and provoke bypass of AUG codons (Fig. 9). This effect will be exacerbated by Ssu$^-$ mutations in eIF1 or eIF1A, which favor the open conformation, producing an even stronger bypass of AUGs with poor context. In contrast, by favoring the closed conformation, Sui$^-$ mutations will mitigate the destabilizing effect of poor context and restore recognition of AUGs in poor context.

It is conceivable that one or more of the initiation factors also plays a direct role in "reading" the sequence context of the start codon. However, the fact that the Sui$^-$ and Ssu$^-$ mutations we analyzed alter the efficiencies of initiation with either start codon mismatches or poor contexts favors the notion that eIF1, eIF1A, and eIF2β, together with AUG and a favorable context, all contribute to the formation or stability of the closed conformation as the means of promoting AUG recognition (Fig. 9) rather than interacting directly with context nucleotides. On the other hand, Pestova et al. reported that in reconstituted mammalian PICs, the −3 context nucleotide (when substituted with 4-thiouridine) could be cross-linked to eIF2α, and that replacing heterotrimeric eIF2 with the eIF2βγ

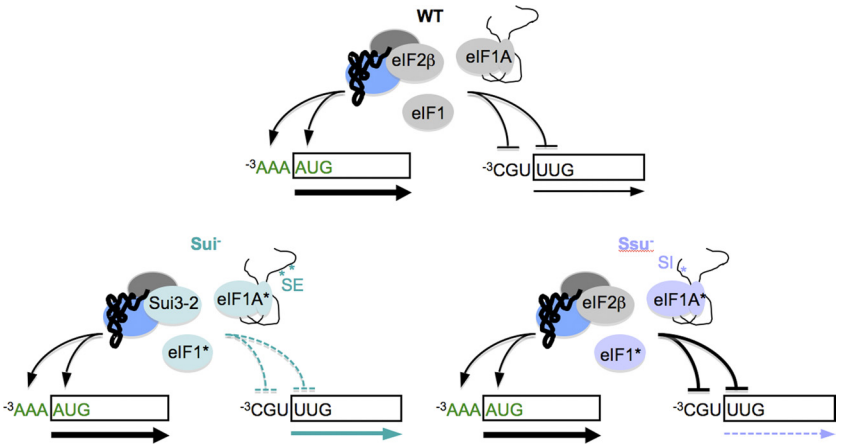



FIG. 9. Specific domains or residues in eIF1, eIF1A and eIF2β discriminate against both poor AUG context and non-AUG start codons to maintain optimal initiation accuracy. In the WT, these initiation factors promote recognition of AUG start codons in optimal context ($^{-3}$AAA) and impede recognition of initiation sites with poor context, such as the $^{-3}$CGU sequence at *SUI1*, or with a non-AUG start codon such as UUG. Sui$^-$ mutations affecting any of these factors render initiation less accurate by diminishing antagonism of start sites with either poor context or a non-AUG start codon. Ssu$^-$ mutations affecting eIF1 or eIF1A render initiation hyperaccurate by increasing antagonism of start sites with either poor context or a non-AUG start codon.

heterodimer (lacking eIF2α) diminished the effect of good context in addition to reducing the efficiency of AUG recognition during 48S assembly (37). Thus, it is possible that interaction of eIF2α with the −3 base helps to stabilize the closed 40S conformation, or the P site binding of Met-tRNA$_i^{Met}$, as a way of promoting selection of AUG codons in good context.

In contrast to the Sui$^-$ mutations in eIF1, eIF1A, and eIF2β, the *SUI5* mutation in eIF5 did not appear to suppress the poor context at *SUI1*, since the expression of eIF1 or *SUI1-lacZ* was not increased in *SUI5* cells. This seems incompatible with the conclusion above that *SUI5* destabilizes the open conformation of the PIC and accelerates GTP hydrolysis by the TC; however, it was shown previously that *SUI5* efficiently rescues initiation with UUG but not with other near-cognates, including AUU, CUG, or GUG, as the *HIS4* start codon (21). In addition, *SUI5* stabilized the closed conformation of 48S PICs reconstituted with UUG but not AUU start codons (29). Thus, *SUI5* might stabilize the closed complex only in response to the UUG-anticodon mismatch and be unable to compensate for the destabilizing effect of poor context. The *SUI3-2* mutation in eIF2β, by contrast, increases initiation from nearly all functional near-cognate triplets *in vivo* (21, 46) in addition to its ability (shown here) to overcome poor context at *SUI1*.

It was demonstrated recently that eIF1 discriminates against poor AUG context in mammalian cells, such that eIF1 overexpression specifically repressed by a factor of ~5 the expression of luciferase reporters lacking both critical residues of the optimal "Kozak" consensus (purine at −3 and G at +4) (24) and repressed by an order of magnitude a reporter gene containing the poor consensus found at the native gene encoding eIF1 (*EIF1*) (22). This repressive function underlies the robust autoregulation of *EIF1* expression in mammalian cells. In comparing these results to ours, it appears that eIF1 is more effective in discriminating against poor context in mammalian cells than in yeast, as the *SUI1-lacZ* reporter with native context was repressed by only a factor of 2 in response to high-level eIF1 overexpression in yeast. As a consequence, eIF1 is substantially overexpressed in cells harboring the hc *SUI1* plasmid (with the native, poor context), whereas overexpression of eIF1 in mammalian cells was achieved only by introducing additional copies of the *EIF1* gene modified to contain the optimal Kozak consensus sequence (22). Moreover, replacing the native *EIF1* context with the optimal Kozak consensus increased reporter expression by a factor of 4, while the comparable replacement in yeast increased *SUI1-lacZ* expression by only a factor of ~2. These observations are consistent with previous results indicating that AUG context has a smaller effect on translation efficiency in yeast (2, 10) versus mammalian cells (23, 24). Nevertheless, it is now clear that in yeast, as in mammals, eIF1 can play an important regulatory role in modulating the expression of genes containing either a poor AUG context or a non-AUG start codon. Moreover, since the ability of eIF1 to autoregulate translation operates in fungi as well as mammals, it should be regarded as the conserved, eukaryotic equivalent of the autoregulatory mechanism first described for IF3 in bacteria.

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