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Genetics

Mutations in the structural genes for eukaryotic initiation factors 2α and 2β of *Saccharomyces cerevisiae* disrupt translational control of *GCN4* mRNA

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ABSTRACT **The *SUI2* and *SUI3* genes of *Saccharomyces cerevisiae* encode the α and β subunits, respectively, of translation initiation factor eIF-2 (eukaryotic initiation factor 2). Previously isolated mutations in these genes restore expression from *his4* mutant alleles lacking an ATG initiation codon. The *SUI* mutations also lead to increased levels of *HIS4* mRNA. We show that the latter phenotype exists because the *SUI* mutations elevate expression of *GCN4*, an activator of *HIS4* transcription. Increased *GCN4* expression in the *SUI* mutants occurs independently of the *GCN2* and *GCN3* gene products that are normally required to stimulate translation of *GCN4* mRNA under conditions of amino acid starvation. Derepression of *GCN4* expression in the *SUI* mutants requires the multiple AUG codons in the leader of the *GCN4* transcript that normally mediate its translational control by amino acid availability. In these respects, the *SUI* mutations resemble mutations in *GCD* genes whose products function as translational repressors of *GCN4*. Thus, in addition to its general role in AUG start codon selection, eIF-2 appears to be an important factor in *GCN4* translational control. We also show that deletion of *GCN3* in *sui2-1* strains is lethal, suggesting that GCN3 contributes to eIF-2α function in addition to its role as a translational activator of *GCN4*.**

Translation initiation in *Saccharomyces cerevisiae* follows the "scanning" mechanism, according to which an initiation complex forms at the capped 5′ end of the mRNA and advances in the 3′ direction until encountering an AUG codon, whereupon translation begins (1–5). Reversion analysis of initiation codon mutations at the *HIS4* gene has identified at least one factor that might mediate a specific interaction between the initiation complex and the AUG start codon. Mutations isolated in either the *SUI2* or *SUI3* gene allow translation of *HIS4* mRNA to begin at a UUG codon in the absence of the normal AUG start codon. The 5′-proximal UUG codon in *HIS4* mRNA is used preferentially as the start site, indicating that initiation at UUG codons in the *SUI* mutants follows the scanning mechanism. Of great interest is the finding that the *SUI2* and *SUI3* gene products are the α and β subunits, respectively, of eukaryotic initiation factor eIF-2 in yeast (6, 7). These results suggest that eIF-2 has an important function in initiation site selection during the scanning process. The fact that the known *SUI3* mutations alter amino acids in the vicinity of a "zinc finger" motif suggests that eIF-2β may influence start-site selection by direct interaction with the mRNA.

In addition to their effects on translation initiation at *HIS4*, *SUI2* and *SUI3* mutations lead to constitutively derepressed amounts of *HIS4* mRNA (8). Normally, derepression of *HIS4* transcription is observed only under conditions of amino acid starvation. This response is mediated by the GCN4 protein, a transcriptional activator of amino acid biosynthetic genes in several different pathways. Expression of *GCN4* is itself regulated by amino acid availability, but at the level of translation initiation. GCN4 protein synthesis is blocked under normal growth conditions by upstream open reading frames (URFs) present in the leader of *GCN4* mRNA. Trans-acting factors encoded by *GCD* genes are required for translational repression by the URFs. Consequently, *gcd* mutations lead to derepressed *GCN4* expression, which in turn stimulates transcription of structural genes like *HIS4* that are under GCN4 control. GCN1, GCN2, and GCN3 are positive factors required for increased translation of *GCN4* mRNA in starvation conditions. These factors are believed to function indirectly by negative regulation of *GCD* gene products, because the nonderepressible phenotype that results from mutations in *GCN1*, *GCN2*, or *GCN3* is overridden by *gcd* mutations. Thus, a pathway of positive and negative regulatory factors functions to couple the rate of translation of *GCN4* mRNA to the availability of amino acids (Fig. 1) (reviewed in ref. 9). The precise roles of the *GCN* and *GCD* factors in *GCN4* translational control have not been established.

The fact that mutations in *SUI2* and *SUI3* alter the specificity of translation initiation at *HIS4*, and also resemble *gcd* mutations in derepressing *HIS4* transcription, suggested to us that eIF-2 may have an important function in *GCN4* translational control. To pursue this possibility, we determined the extent to which the phenotypes of mutations in *SUI2* and *SUI3* correspond to the phenotypes of known *gcd* mutations. First, if SUI2 and SUI3 function like GCD factors in the general control regulatory pathway, then derepression of amino acid biosynthetic enzymes in *SUI* mutants should require a functional *GCN4* gene. By contrast, enzyme derepression in the *SUI* mutants should be independent of the *GCN2* and *GCN3* gene products (Fig. 1). Second, *GCN4* expression should be constitutively derepressed in the *SUI* mutants, and this increased expression should require the multiple URFs in *GCN4* mRNA that normally mediate its translational control. Our results show that the *sui2-1* and *SUI3-2* mutant alleles resemble *gcd* mutations in all these respects, leading us to propose that modulation of eIF-2 function in response to amino acid availability may be an important aspect of *GCN4* translational control.

MATERIALS AND METHODS

Construction of *gcn* Chromosomal Deletions. The *SUI* mutant strains used in these constructions, TD304-10B and H1022, contain the *sui2-1* and *SUI3-2* mutations, respectively, and were derived from 117-8AR-20 (7) and 117-1AR-7 (6). The *SUI3-2* allele is dominant for suppression of the His⁻

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Abbreviations: eIF-2, eukaryotic initiation factor 2; URF, upstream open reading frame; 3-AT, 3-aminotriazole; 5-MT, 5-methyltryptophan; 5-FT, 5-fluorotryptophan.

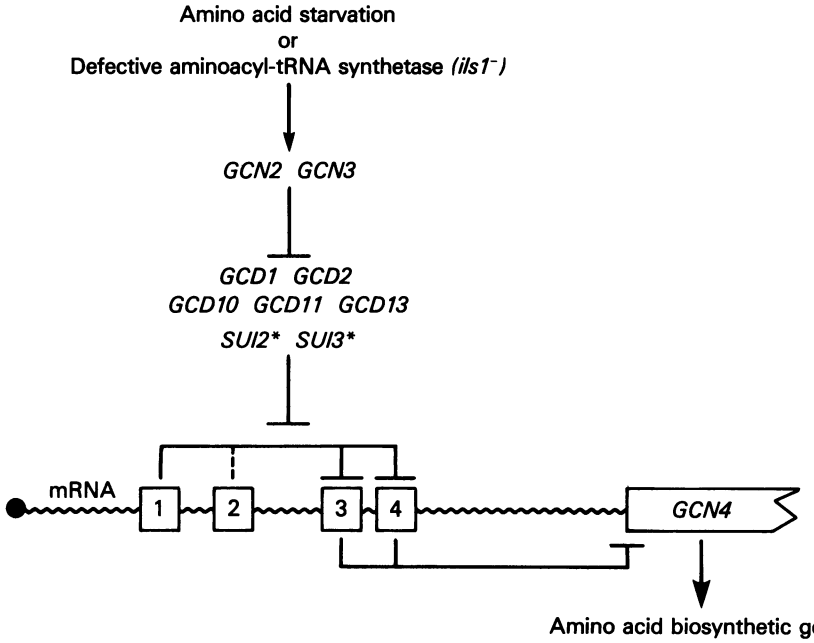

FIG. 1. Pathway of regulatory factors involved in translational control of *GCN4* expression. Arrows indicate stimulatory interactions; bars depict inhibitory interactions. The four URFs in the *GCN4* mRNA leader are shown as numbered boxes. URF-3 and -4 function as the major translational barriers to *GCN4* expression in repressing conditions. The inhibitory effect of these sequences is reduced in derepressing conditions by URF-1 and -2, the greater effect being exerted by URF-1. The antagonistic interaction between the URFs is modulated by the trans-acting positive (*GCN*) or negative (*GCD* and *SUI*) factors in response to the abundance of aminoacylated tRNA. The positions of *SUI2* and *SUI3* in the pathway (*) were determined in this study; the other trans-acting factors were analyzed previously (reviewed in ref. 9). The products of *GCN1* and *GCD3–GCD7* are also thought to function in this regulatory pathway (9); however, they were not included here because it has not been shown directly that they regulate *GCN4* expression at the translational level (9).

phenotype of the initiation codon mutation *his4-303*; *sui2-1* is recessive for this suppressor phenotype. These two *SUI* mutations were chosen for analysis because their effects on translation of *HIS4* mRNA have been extensively characterized at the molecular level (6, 7) and they are known to increase *HIS4* expression at the transcriptional level (8). Deletions of *GCN* genes were constructed in strains described in Table 1 by the one-step gene replacement technique (10) and were verified by DNA blot-hybridization analysis. The *gcn2::LEU2* allele lacks a 0.45-kilobase (kb) *Bgl* II fragment in the *GCN2* protein-coding sequences (11), the *gcn4::LEU2* allele lacks sequences between positions +88 and +1092 relative to the 5′ end of *GCN4* mRNA (12), and the *gcn3::LEU2* construct lacks a 0.78-kb *Hin*dIII/*Cla* I fragment, representing 60% of the protein-coding sequences (13). All three deletion alleles contain a 2.8-kb *Bgl* II *LEU2* fragment inserted in place of the deleted sequences.

Table 1. *sui2-1* and *SUI3-2* derepress tryptophan biosynthetic enzymes independent of GCN2 but dependent on GCN4 function

Strain	Relevant genotype	Growth on 5-MT	Growth on 5-FT
1	*SUI GCN*	+	−
2	*SUI gcn2::LEU2*	−	−
3	*SUI gcn4::LEU2*	−	−
4	*SUI GCN gcd2-1*	+	+
5	*SUI gcn2::LEU2 gcd2-1*	+	+
6	*sui2-1 GCN*	+	+
7	*sui2-1 gcn2::LEU2*	+	+
8	*sui2-1 gcn4::LEU2*	−	−
9	*SUI3-2 GCN*	+	+
10	*SUI3-2 gcn2::LEU2*	±	±
11	*SUI3-2 gcn4::LEU2*	−	−

Strains 2 (H1069) and 3 (H1080) are *gcn::LEU2* transformants of strain 1 (H4) (*MATa leu2-3 leu2-112 ura3-52*). Likewise, strain 5 (H1043) is a transformant of strain 4 (H952) (*MATα leu2-3 leu2-112 ura3-52 gcd2-1*); strains 7 (H1066) and 8 (H1079) are transformants of strain 6 (TD304-10B) (*MATa leu2-3 leu2-112 ura3-52 his4-303 sui2-1*); strains 10 (H1067) and 11 (H1078) are transformants of strain 9 (H1022) (*MATα leu2-3 leu2-112 ura3-52 his4-303 SUI3-2*). Sensitivity to 5-MT or 5-FT was scored qualitatively as the size of colonies formed 3 days after plating single cells on 5-MT medium or the amount of growth seen in replica-printed patches of cells 2–3 days after printing to 5-FT medium, with 5-MT or 5-FT present at 0.5 mM.

Demonstration That *SUI* Mutations Are Suppressors of the Nonderepressible Phenotype Associated with *gcn2::LEU2*. The *gcn2::LEU2 sui2-1 his4-303* and *gcn2::LEU2 SUI3-2 his4-303* derivatives of TD304-10B and H1022 were each crossed to a *gcn2::LEU2 SUI his4-303* strain and the sporulated diploids were subjected to tetrad analysis. We observed 2+:2− segregation for the Sui⁻ phenotype in 10 complete tetrads and every Sui⁻ ascospore contained a suppressor of the 3-aminotriazole-sensitive (3-AT^s) phenotype associated with *gcn2::LEU2*. (3-AT is an inhibitor of histidine biosynthesis to which *gcn* mutants are sensitive.) *sui2-1* was scored by temperature-sensitive growth on rich medium (yeast extract/peptone/dextrose). *SUI3-2* was scored by suppression of the His⁻ phenotype of *his4-303*. Because *his4-303* mutants cannot grow on 3-AT medium, 3-AT sensitivity was scored after first mating all haploid segregants to a *gcn2::LEU2 SUI HIS4* strain. Suppression of the 3-AT^s phenotype of *gcn2::LEU2* was evident in the resulting *gcn2::LEU2/gcn2::LEU2* (*sui2-1* or *SUI3-2*)/*SUI his4-303/HIS4* diploids because the *sui2-1* and *SUI3-2* mutations are both semidominant for suppression of the nonderepressible phenotype associated with *gcn2::LEU2*.

Measurement of *GCN4–lacZ* Expression. Plasmids p180, p226, and p227 are low-copy replicating plasmids containing *GCN4–lacZ* translational fusions with different combinations of URFs in the mRNA leader. Particular URFs were removed from the fusions present in p226 and p227 by single-base-pair substitutions in their ATG start codons (14), none of which introduces a new UUG codon into the mRNA leader. These plasmids were introduced into yeast strains by the lithium acetate method (15). β-Galactosidase activity was assayed in the transformants as described (16) after growing cells for 6 hr in SD minimal medium supplemented with the required nutrients (17) or for 6 hr in the same medium supplemented with 10 mM 3-AT to cause histidine starvation (Table 2) or 0.5 mM 5-methyltryptophan to induce tryptophan starvation (Table 3). Steady-state levels of *GCN4–lacZ*, *GCN4*, and *PYK* mRNAs were measured by blot-hybridization analysis as described (18).

RESULTS

Derepression of Tryptophan Biosynthetic Enzymes in *SUI2* and *SUI3* Mutants Requires GCN4 but Is Independent of GCN2. We began our studies by determining whether dere-

Table 2. *sui2-1* and *SUI3-2* derepress *GCN4* expression independent of GCN2 function

Strain	Genotype	*GCN4–lacZ* enzyme activity, units I R	I DR	II R	II DR	Growth on 3-AT
1	*SUI GCN*	14	130	12	100	+
2	*SUI gcn2::LEU2*	8	19	11	20	−
3	*SUI GCN gcd2-1*	640	700	ND	ND	+
4	*SUI gcn2::LEU2 gcd2-1*	310	280	ND	ND	+
5	*sui2-1 GCN*	270	240	160	160	+
6	*SUI3-2 GCN*	120	200	140	170	+
7	*sui2-1 gcn2::LEU2*	250	220	130	120	+
8	*SUI3-2 gcn2::LEU2*	92	79	82	84	+

Strains 3 and 4 are H952 and H1043, respectively, described in Table 1. All other strains were derived from crosses between the *gcn2::LEU2* derivatives of TD304-10B or H1022 and AGH601-1B (*MATα ura3-52 ino1*) or TD28 (*MATa ura3-52 ino1*). Expression of p180, a low-copy plasmid containing the *GCN4–lacZ* fusion with the wild-type mRNA leader, was measured in two different strains of each genotype (I and II), grown in minimal medium (repressing, R) or in minimal medium containing 10 mM 3-AT to cause histidine starvation (derepressing, DR). Values shown are averages calculated from two or three independent measurements, each differing from the mean value by 30% or less. ND, data not determined. Strains were tested for sensitivity to 3-AT to determine their ability to derepress histidine biosynthetic enzymes by replica-printing patches of cells to minimal medium containing 30 mM 3-AT, 40 mM L-leucine, and all amino acids except histidine at 2 g/liter. Growth was scored after 2–3 days.

pression of amino acid biosynthetic enzymes in the *SUI* mutants is dependent on GCN4 and GCN2, two positive regulators in the general control system. *gcn4::LEU2* and *gcn2::LEU2* deletion alleles were introduced in place of the corresponding wild-type *GCN* genes in *sui2-1* and *SUI3-2* mutants and in a wild-type *SUI* strain. The resulting strains were tested for their ability to derepress tryptophan biosynthetic enzymes (one of several pathways subject to the general control) by measuring the growth rate in the presence of two tryptophan analogues. In otherwise wild-type strains, *gcn2* and *gcn4* mutations confer increased sensitivity to 5-methyltryptophan (5-MT), an inhibitor of tryptophan biosynthesis, because these mutations impair derepression of the tryptophan pathway under starvation conditions. *gcd* mutations restore tryptophan enzyme derepression in *gcn2* mutants and thereby overcome 5-MT sensitivity (19). A different analogue, 5-fluorotryptophan (5-FT), competes with tryptophan for incorporation into proteins but does not cause tryptophan starvation or enzyme derepression. Because *gcd* mutations lead to constitutively elevated tryptophan enzymes and higher tryptophan pools, *gcd* and *gcd gcn2* double mutants are more 5-FT resistant than wild-type cells or *gcn2* single mutants (19). *gcn4* mutants are unconditionally defective for derepression and hence show sensitivity to both analogues in the presence or absence of *gcd* mutations (19). Thus, wild-type, nonderepressible (Gcn^-) and constitutively derepressed (Gcd^-) strains can be easily distinguished by their sensitivity to these compounds. Moreover, the degree of sensitivity correlates well with measurements of amino acid biosynthetic enzyme levels (19) and *GCN4–lacZ* expression (9) in strains containing these regulatory mutations.

The data in Table 1 show that *sui2-1* and *SUI3-2* behaved like known *gcd* mutations in causing constitutive derepression of tryptophan biosynthetic enzymes (resistance to both 5-MT and 5-FT) in both *GCN2* and *gcn2::LEU2* strains. The derepressed phenotype observed in the *sui2-1 gcn2::LEU2* and *SUI3-2 gcn2::LEU2* double mutants indicates that *sui2-1* and *SUI3-2* overcome the requirement for GCN2-positive regulatory function for increased expression of tryptophan enzymes (Fig. 1). The *sui2-1* mutation appears to completely restore enzyme derepression in the *gcn2::LEU2* strain; by comparison, derepression in the *SUI3-2 gcn2::LEU2* strain was somewhat reduced from its level in the *SUI3-2 GCN2* parent strain. In contrast to these results, no derepression was evident in either *SUI* mutant containing the *gcn4::LEU2* allele, the same result observed previously for all known *gcn4 gcd* double mutants (9). Thus, as in *gcd* mutants, derepression of tryptophan enzymes in response to the *SUI* mutations is mediated by GCN4 (Fig. 1). Meiotic analysis was used to confirm that the 5-MT-resistant phenotype of the *sui2-1*

Table 3. Efficient derepression of *GCN4–lacZ* in *sui2-1* and *SUI3-2* mutants requires multiple upstream AUG codons in the *GCN4* mRNA leader

Strain	Genotype	*GCN4–lacZ* enzyme activity, units: p180 (URF-1 to -4) R	p180 DR	p226 (URF-4 only) R	p226 DR	p227 (no URFs) R	p227 DR
1	*SUI GCN*	13	75	8	21	1100	980
2	*SUI GCN gcd2-1*	1100	570	82	41	1900	1500
3	*sui2-1 GCN*	200	190	23	17	1000	970
4	*SUI3-2 GCN*	130	210	18	32	950	780
5	*SUI gcn2::LEU2*	3	3	5	8	760	580
6	*SUI gcn3::LEU2*	11	21	ND	ND	970	780
7	*SUI gcn2::LEU2 gcd2-1*	340	310	58	49	1200	1100
8	*sui2-1 gcn2::LEU2*	240	200	36	32	1400	1300
9	*SUI3-2 gcn2::LEU2*	110	92	17	16	790	790
10	*SUI3-2 gcn3::LEU2*	120	150	16	18	860	790

Strain 5 (H1143) is a *gcn2::LEU2* transformant of strain 1 (76-3D, *his4-303 ura3-52 leu2-3 leu2-112*). Likewise, strain 6 (H1070) is a transformant of H4; strain 7 (H1043) is a transformant of strain 2 (H952); strain 8 (H1066) is a transformant of strain 3 (TD304-10B); and strain 9 (H1067) and strain 10 (H1068) are transformants of strain 4 (H1022). Units of β-galactosidase were measured in transformants containing either p180, p226, or p227, as described in Table 2, except that 5-MT was added to induce tryptophan starvation. (Note that 5-MT is routinely less effective as a derepressing agent than 3-AT.) Plasmids p226 and p227 are identical to p180 (described in Table 2) except in containing point mutations in the first three (p226) or all four (p227) ATG codons of the *GCN4* mRNA leader (14). The values shown are averages of two or three independent measurements made in repressing (R) or derepressing (DR) conditions. ND, data not determined.

gcn2::*LEU2* and *SUI3-2 gcn2*::*LEU2* double mutants was the result of the *SUI* mutations rather than of cryptic *gcd* mutations (see *Materials and Methods*). These results show that the *sui2-1* and *SUI3-2* mutations exhibit the same interactions with *gcn2* and *gcn4* mutations that were described previously for *gcd* mutations (reviewed in ref. 9). Based on these findings, the eIF-2α and -2β subunits are expected to function in the general control regulatory pathway at the same position proposed for *GCD* regulatory factors (Fig. 1).

***SUI2* and *SUI3* Mutations Derepress *GCN4–lacZ* Expression at the Translational Level.** It was anticipated that derepression of tryptophan enzymes occurred in the *SUI* mutants as the result of increased synthesis of GCN4 protein, a transcriptional activator of *TRP* genes. This prediction was tested by measuring β-galactosidase activity expressed from a *GCN4–lacZ* fusion in *SUI* mutant and wild-type strains. *GCN4–lacZ* enzyme activity derepresses ≈10-fold in response to histidine starvation in wild-type cells, and a *gcn2* mutation, for example, impairs this derepression response. Known *gcd* mutations result in constitutive derepression of *GCN4–lacZ* expression at levels between 10- and 100-fold higher than the wild-type repressed level, either in the presence or absence of a functional *GCN2* gene (refs. 20 and 21; Table 2). The *sui2-1* and *SUI3-2* mutations lead to 10- to 20-fold increases in *GCN4–lacZ* expression in normally repressing conditions (Table 2). In addition, this derepression was maintained after *GCN2* was replaced by *gcn2*::*LEU2*, occurring at levels 6- to 10-fold higher than the wild-type repressed level and 10- to 20-fold higher than that seen in *SUI gcn2*::*LEU2* strains. Thus, like known *gcd* mutations, *sui2-1* and *SUI3-2* increase *GCN4* expression independent of *GCN2* positive regulatory function.

We examined the effects of the *SUI* mutations on the steady-state amounts of both authentic *GCN4* mRNA and *GCN4–lacZ* transcripts produced from plasmid p180. A 2-fold increase in the amounts of both transcripts was seen in a *sui2-1* mutant compared to a *SUI* strain under repressing conditions (Fig. 2). In the same circumstances, 15-fold greater *GCN4–lacZ* enzyme activity was expressed in the *sui2-1* mutant compared to the wild-type strain (compare data for p180 transformants of strains 1 and 3 under repressing conditions; Table 3). Little or no difference was seen between the levels of *GCN4–lacZ* mRNA in the *SUI3-2* and *SUI* strains (Fig. 2). In the same conditions, *GCN4–lacZ* enzyme

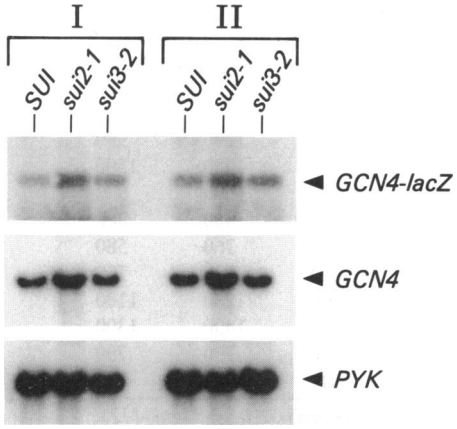

FIG. 2. RNA blot-hybridization analysis of *GCN4* and *GCN4–lacZ* transcripts in total RNA isolated from two independent sets of p180 transformants of *sui2-1* strain TD304-10B, *SUI3-2* strain H1022, and *SUI* strain 76-3D, grown under the same repressing conditions described in Tables 2 and 3. RNA blots were hybridized with radiolabeled *GCN4* or *PYK* DNA fragments described previously (14). The *GCN4* probe hybridizes to *GCN4–lacZ* transcripts in addition to authentic *GCN4* mRNA.

activity was 10-fold greater in the *SUI3-2* mutant compared to wild-type (compare data for p180 transformants of strains 1 and 4 in Table 3). These results suggest that the *sui2-1* and *SUI3-2* mutations increase *GCN4* expression primarily at the translational level. Additional support for this conclusion is presented in the next section.

Derepression of *GCN4–lacZ* Enzyme Activity in the *SUI* Mutants Requires Multiple Upstream AUG Codons in the *GCN4* mRNA Leader. Translational control of *GCN4* mRNA is mediated by four URFs present in the mRNA leader. URF-3 and -4 both function as strong translational barriers to *GCN4* expression. In fact, URF-4 is sufficient to repress *GCN4* expression to the low level observed in nonstarvation conditions when all four URFs are present in the leader. By contrast, URF-1 (and to a lesser extent URF-2) is required under derepressing conditions to efficiently overcome the translational block at URF-3 to -4 (Fig. 1). Accordingly, mutations that remove URF-1 to -3 and leave only URF-4 in the mRNA leader diminish *GCN4–lacZ* expression, particularly in amino acid-starved wild-type cells or in *gcd* mutants (refs. 14 and 22; Table 3). Removal of URF-1 to -3 by single-base-pair substitutions substantially reduced the derepressing effect of the *sui2-1* and *SUI3-2* mutations on *GCN4–lacZ* expression (compare strain 1 with strains 3 and 4 and strain 5 with strains 8 and 9 in Table 3). These results indicate that the *SUI* mutations require the 5′-proximal URFs to efficiently derepress *GCN4* expression in the presence of URF-4 (Table 3). The *SUI* mutations further resemble *gcd* mutations in producing little or no increase in *GCN4–lacZ* expression when all four URFs are missing from the mRNA leader (Table 3). Because the *SUI* mutations require the same combination of URFs to increase *GCN4* expression that was established previously for *gcd* mutations, it appears that SUI2 and SUI3 function like the GCD factors in controlling *GCN4* expression by influencing the regulatory functions of the URFs. Neither *sui2-1* nor *SUI3-2* is allelic to mutations in any of the five *GCD* genes shown in Fig. 1 (*GCD1*, *GCD2*, and *GCD10–GCD13*; N.P.W. and A.G.H., unpublished observations).

A *sui2-1 gcn3*::*LEU2* Double Mutation Leads to Inviability. All attempts to replace the wild-type *GCN3* gene with *gcn3*::*LEU2* in a haploid *sui2-1* strain yielded no transformants. Therefore, we replaced one copy of *GCN3* in a *sui2-1*/*sui2-1 leu2*/*leu2* diploid strain with *gcn3*::*LEU2* and sporulated the diploid transformants. Each of 10 tetrads dissected contained two Leu$^-$ (*GCN3*) spores that grew on rich medium at the same rate as their haploid parents and two spores that failed to form visible colonies or, in rare cases, gave rise to extremely slow-growing Leu$^+$ (*gcn3*::*LEU2*) clones. These findings indicate that *sui2-1 gcn3*::*LEU2* double mutants are inviable. This result is interesting because *gcn3*::*LEU2* is lethal, or reduces the growth rate substantially, in *gcd1* and certain *gcd2* mutants (13, 23). By contrast, deletion of *GCN3* has no detectable effect on growth in otherwise wild-type strains (13) or in the *SUI3-2* mutant. These findings suggest that, at least in a *sui2-1* mutant, *GCN3* interacts with the α subunit of eIF-2 to promote an essential function involved in protein synthesis. The genetic interactions detected between *gcn3*, *gcd1*, and *gcd2* mutations suggest that GCD1 and GCD2 are also involved in this function.

DISCUSSION

***SUI2* and *SUI3* Mutations Alter Interactions Between the URFs That Regulate Translation Initiation at the *GCN4* Start Codon.** The fact that the *sui2-1* and *SUI3-2* mutations alter the specificity of start-site selection at *HIS4* implies that eIF-2 plays an important role in recognition of AUG codons during the scanning process. Given that *GCN4* translational control depends on the recognition of upstream AUG codons, it is of

great interest that mutations affecting eIF-2 function impair this regulatory mechanism. Our results show that the *sui2-1* and *SUI3-2* mutations abolish translational repression of *GCN4*, leading to constitutively derepressed *GCN4* expression independent of amino acid availability. The derepressing effects of these mutations on *GCN4–lacZ* enzyme activity are much greater in the presence of multiple URFs compared to the situation in which URF-4 is present singly or when no URFs are present in the mRNA. Thus, the *SUI* mutations appear to alter the interplay between the 5′-proximal and 3′-proximal URFs that normally regulate the rate of translation initiation at the *GCN4* start codon.

When present alone in the mRNA leader, URF4 reduces *GCN4–lacZ* expression by ≈99% from the high level observed when no upstream URFs are present (14). Apparently, in this situation, most scanning ribosomes initiate at URF-4 and fail to reinitiate at the GCN4 start site downstream. The *SUI* mutations have little effect on *GCN4–lacZ* expression when only URF-4 is present in the leader; therefore, these mutations do not simply cause ribosomes to bypass upstream AUG codons. However, when the 5′-proximal URFs are present upstream from URF-4, the *sui2-1* mutation results in a 10- to 20-fold increase in the number of ribosomes that are able to advance beyond URF-4 and initiate GCN4 protein synthesis. Clearly, recognition of the AUG start codons of both the 5′-proximal and the 3′-proximal URFs remains intact in the *SUI* mutants and is required for the elevated *GCN4* expression observed in these strains. Therefore, it is improbable that the *SUI* mutations derepress *GCN4* expression in the same way that they restore translation of *HIS4* mRNA in the absence of an AUG start codon—i.e., by permitting UUG codons to function more efficiently as translation start sites. Rather, our results suggest that translation of the 5′-proximal URFs (principally URF-1; ref. 14), coupled with altered eIF-2 activity, allows a fraction of ribosomes to either bypass the URF-4 start site or to more efficiently reinitiate at the *GCN4* AUG codon following URF-4 translation.

One way to explain the requirement for URF-1 translation for increased *GCN4* expression in the *SUI* mutants is to propose that translation of this sequence leads to a conformational change in the mRNA that is required in conjunction with altered eIF-2 activity for ribosomes to advance past URF-4. An alternative possibility is that 40S subunits resume scanning after URF-1 translation, but they lack certain factors that associate with 40S subunits only during their interaction with the mRNA 5′ cap. The absence of such factors from initiation complexes that re-form after URF-1 translation might be required for a response to subtle changes in eIF-2 activity that allow reinitiating ribosomes to move beyond URF-4 to the *GCN4* start site.

The *SUI* Mutations Overcome the Requirement for the Positive Regulators GCN2 and GCN3 for Derepression of *GCN4*. The *sui2-1* and *SUI3-2* mutations overcome the requirement for the positive regulators GCN2 and GCN3 for derepression of *GCN4* expression. This result, together with the fact that *SUI* mutations elevate *GCN4* expression only in the presence of the multiple URFs that normally mediate translational activation by GCN2 and GCN3, suggests that these GCN factors function by negative regulation of the *SUI* gene products (Fig. 1). Thus, eIF-2 activity may be modulated by GCN2 and GCN3 according to the availability of amino acids in the cell. Some *gcd* mutations lead to greater derepression of *GCN4–lacZ* expression than that observed for *sui2-1* and *SUI3-2* (21). This difference could indicate that the mutant *SUI* gene products studied here are only partially defective for a function of eIF-2 that is needed to repress translation of *GCN4* mRNA under normal growth conditions. Alternatively, eIF-2 may be only one of several targets of GCN2 and GCN3.

GCN2 contains a domain homologous to known protein kinases, and evidence was presented that *GCN2* encodes or regulates a protein kinase activity that increases under amino acid starvation conditions (11). Substitution of a lysine residue in GCN2 that is invariant among known protein kinases and thought to function in the phosphotransfer reaction inactivates GCN2-positive regulatory function (24). These results suggest that GCN2 stimulates *GCN4* expression under starvation conditions by functioning as a protein kinase. This possibility is interesting in view of the present results because eIF-2 activity is reduced in mammalian cells by increased phosphorylation of the α subunit in response to certain stress conditions, including amino acid starvation (25). Moreover, eIF-2α is subject to phosphorylation in *S. cerevisiae* (7). Perhaps GCN2, or another factor under its control, alters the phosphorylation state of eIF-2α under starvation conditions to modify the mechanism of translation initiation in a way that permits ribosomes to advance beyond the URFs and initiate translation at the GCN4 start site.

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