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Genetic Characterization of the *Saccharomyces cerevisiae* Translational Initiation Suppressors *sui1*, *sui2* and *SUI3* and Their Effects on *HIS4* Expression

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ABSTRACT

Saccharomyces cerevisiae strains containing mutations of the *HIS4* translation initiation AUG codon were studied by reversion analysis in an attempt to identify components of the translation initiation complex that might participate in initiation site selection during the scanning process. The genetic characterization of these revertants identified three unlinked suppressor loci: *SUI1*, *SUI2* and *sui3*, which when mutated restored the expression of the *HIS4* allele despite the absence of the AUG initiator codon. Both *sui1* and *sui2* are recessive and cause temperature-sensitive growth on enriched medium. The temperature-sensitive phenotype and the ability to restore *HIS4* expression associated with either *sui1* or *sui2* mutations cosegregate in crosses. *SUI3* mutations are dominant and do not alter the thermal profile for growth. None of the mutations at the three loci suppresses known frameshift, missense or nonsense mutations. Each is capable of suppressing the nine different point mutations of the initiator codon at *HIS4* or *HIS4-lacZ* as well as a two base change (ACC) and a three base deletion of the AUG codon, suggesting that the site of suppression resides outside the normal initiator region. *sui1* and *sui2* suppressor mutations were mapped to chromosomes *XIV* and *X*, respectively. Suppression by *sui1*, *sui2* and *SUI3* mutations results in 14-, 11- and 47-fold increases, respectively, relative to isogenic parent strains, in the expression of a *HIS4* allele lacking the initiator AUG codon. Part of this increase in the *HIS4* expression by *sui2* and *SUI3* can be attributed to increases of *HIS4* mRNA levels, presumably mediated by perturbation of the general amino acid control system of yeast.

A model has been proposed for initiation of protein synthesis in eukaryotes, in which the ternary complex, composed of eIF-2.GTP.tRNA$_i^{met}$, binds to the 40S ribosomal subunit, which in turn binds the 5′ end of the message, and migrates in the 5′ to 3′ direction until an AUG codon is found, where initiation occurs (Kozak 1978, 1980). This model has been corroborated in *Saccharomyces cerevisiae* by mutational analysis at the *CYC1* locus (Sherman and Stewart 1982), and at the *HIS4* locus (Cigan, Pabich and Donahue 1988; Donahue and Cigan 1988). It seems that, by virtue of its 5′ position, an AUG is utilized as the site of initiation. Thus, the start site selection mechanism proposed for eukaryotes places the AUG codon and its interaction with the initiator tRNA$_i^{met}$ as a fundamental event in this process. Indeed, it has been demonstrated in the yeast system that ribosomal recognition of a start codon is mediated by the initiator tRNA (Cigan, Feng and Donahue 1988). However, given the complex nature of the translation initiation process, it is conceivable that, in addition to tRNA$_i^{met}$, other components may participate in the start site selection process.

In order to identify other components of the initiation complex that may be important in defining start site selection in eukaryotes, we initiated a genetic analysis of the translation initiation process in *S. cerevisiae*. It has been demonstrated previously that mutations of the AUG initiator codon at *HIS4* result in the ability of the ribosome to bypass the early *HIS4* coding region, resulting in a His$^-$ phenotype (Donahue and Cigan 1988). By selecting for *HIS4* function (His$^+$), revertants of these strains that no longer allow the ribosome to bypass but now initiate at this mutant initiator region should be identified. One type of His$^+$ revertant expected is a result of restoration of the AUG codon at *HIS4*. However, His$^+$ revertants might carry mutations in genes that encode *trans*-acting factors that alter and therefore function in mediating ribosomal recognition of the initiator region during the scanning process. External suppressor mutations were identified by their ability to *trans*-activate two copies of the mutant message, one from the *HIS4* locus, and a second from a translational fusion be-

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TABLE 1

***Saccharomyces cerevisiae* strains**

Strain[a]	Genotype
A) Strains used for reversion analysis	
CUG initiator codon mutation	
115-8C	*MATα his4-302 ino1-13 ura3-52::his4*(CUG)-*lacZ* (Ura^+)
115-10C	*MAT***a** *his4-302 ura3-52::his4*(CUG)-*lacZ* (Ura^+)
116-1C	*MATα his4-302 ino1-13 ura3-52::his4*(CUG)-*lacZ* (Ura^+)
GUG initiator codon mutation	
125-2B	*MAT***a** *his4-305 ura3-52::his4*(GUG)-*lacZ* (Ura^+)
125-5B	*MAT***a** *his4-305 ino1-13 ura3-52::his4*(GUG)-*lacZ* (Ura^+)
124-3D	*MATα his4-305 ino1-13 ura3-52::his4*(GUG)-*lacZ* (Ura^+)
ACG initiator codon mutation	
114-2A	*MAT***a** *his4-301 ino1-13 ura3-52::his4*(ACG)-*lacZ* (Ura^+)
114-4A	*MATα his4-301 ura3-52::his4*(ACG)-*lacZ* (Ura^+)
114-7B	*MAT***a** *his4-301 ura3-52::his4*(ACG)-*lacZ* (Ura^+)
114-7C	*MATα his4-301 ino1-13 ura3-52::his4*(ACG)-*lacZ* (Ura^+)
123-3A	*MAT***a** *his4-301 ura3-52::his4*(ACG)-*lacZ* (Ura^+)
123-3B	*MATα his4-301 ino1-13 ura3-52::his4*(ACG)-*lacZ* (Ura^+)
123-4A	*MATα his4-301 ura3-52::his4*(ACG)-*lacZ* (Ura^+)
JJ2	*MAT***a** *his4-301 ino1-13 ura3-52*
AUU initiator codon mutation	
117-1A	*MAT***a** *his4-303 ino1-13 ura3-52::his4*(AUU)-*lacZ* (Ura^+)
117-8A	*MATα his4-303 ura3-52::his4*(AUU)-*lacZ* (Ura^+)
117-9A	*MAT***a** *his4-303 ura3-52::his4*(AUU)-*lacZ* (Ura^+)
118-2C	*MATα his4-303 leu2-3,-112 ura3-52::his4*(AUU)-*lacZ* (Ura^+)
JJ6	*MAT***a** *his4-303 ura3-52*
ACC initiator codon mutation	
113-2A	*MAT***a** *his4-300 ino1-13 ura3-52::his4*(ACC)-*lacZ* (Ura^+)
B) Wild-type and mutant *HIS4* strains used for genetic tests	
Wild-type *HIS4* or *HIS4-lacZ*	
TD28	*MAT***a** *ura3-52 ino1-13*
6288-8C	*MATα leu2-3,-112*
105-3A	*MATα his4-401 leu2-3,-112 ino1-13 ura3-52::HIS4* (AUG)-*lacZ* (Ura^+)
AUA initiator codon mutation	
69-4C	*MATα his4-619 leu2-3,-112*
TD49	*MATα his4-619 ura3-52*
TD50	*MAT***a** *his4-619 ura3-52*
AUU initiator codon mutation	
76-2B	*MAT***a** *his4-303 leu2-3*
76-3D	*MATα his4-303 ura3-52 leu2-3*
76-8D	*MAT***a** *his4-303 ura3-52 leu2-3*
77-1A	*MATα his4-303 ura3-52*
77-1C	*MAT***a** *his4-303 ura3-52*
AUC initiator codon mutation	
78-1A	*MATα his4-304 ura3-52*
79-6A	*MAT***a** *his4-304 ura3-52*
79-9C	*MATα his4-304 ura3-52 ino1-13*
ACG initiator codon mutation	
73-2C	*MAT***a** *his4-301 leu2-3*
73-3B	*MAT***a** *his4-301 ura3-52 leu2-3*
73-16D	*MATα his4-301 leu2-3 ino1-13*
185-3D	*MATα his4-301 trp1*
222-10A	*MAT***a** *his4-301 ura3-52*
GUG initiator codon mutation	
81-10C	*MATα his4-305 ura3-52 leu2-3*
219-1B	*MAT***a** *his4-305 ura3-52*
219-3B	*MATα his4-305 ura3-52*

[a] Yeast strains were from this laboratory collection and derivatives from this work except: 893, 894, A298-61D, A298-65C, 1412, A256-99A, A236-57B, A193-16C, A121-3A, 1327, 1554, and 1555 from M. CULBERTSON; CSH-87L and CSH-89L from R. ESPOSITO; LR663-1D from K. TATCHELL and AH2, 9436-3B, 9436-10D, 6288-8C and 5470-1C from G. R. FINK. Yeast strains AH2, 5470-1C and TD5 contain the *his4* alleles *his4-519* and *his4-712* which have been shown to contain the +1 frameshift mutations GGG → GGGG and CCU → CCCU, respectively (DONAHUE, FARABAUGH and FINK, 1982). Each mutation at *HIS4* have been demonstrated to be suppressible by glycine (GABER and CULBERTSON 1984) and proline inserting frameshift suppressors (WINEY *et al.* 1989) Yeast strains AGH1 and AGH3 contain the UAG nonsense suppressible alleles *met8-1* and *trp1-1*, the UAA nonsense suppressible alleles *lys1-1* and *arg4-17* and the UGA nonsense suppressible allele *leu2-2* and were obtained from A. HINNEBUSCH.

Strain[a]	Genotype
UUG initiator codon mutation	
167-4C	*MATα his4-306 ura3-52*
167-6B	*MAT***a** *his4-306 ura3-52*
CUG initiator codon mutation	
75-1B	*MAT***a** *his4-302 ura3-52*
75-6D	*MATα his4-302 ura3-52 leu2-3*
75-8A	*MATα his4-302 ura3-52*
234-7B	*MATα his4-302 ura3-52 leu2-3,-112*
ACC initiator codon mutation	
71-1B	*MAT***a** *his4-300 ura3-52 leu2-3*
71-5A	*MATα his4-300 ura3-52*
71-8B	*MATα his4-300 ura3-52 leu2-3*
232-10D	*MAT***a** *his4-300 ura3-52*
Deletion of the initiator AUG	
188-5B	*MAT***a** *his4-307 ura3-52*
188-5D	*MATα his4-307 ura3-52 ino1-13*
189-4D	*MATα his4-307 ura3-52*
Deletion of the *HIS4* locus	
TD77	*MAT***a** *his4-401 ura3-52 ino1-13*
44-1B	*MATα his4-401 leu2-3,-112*
44-5A	*MATα his4-401 ura3-52 leu2-3,-112 ino1-13*
44-22B	*MAT***a** *his4-401 ura3-52 leu2-3,-112 ino1-13*
45-3B	*MATα his4-401 ura3-52 leu2-3,-112*
45-11D	*MAT***a** *his4-401 ura3-52*
65-18B	*MAT***a** *his4-401 ura3-1,-3 leu2-3,-112*
C) Nonsense and frameshift mutations	
AH2	*MAT***a** *his4-519 leu2-3,-112*
5470-1C	*MATα his4-712 leu2-3*
TD5	*MAT***a** *his4-712 ura3-52*
AGH1	*MATα met8-1 trp1-1 lys1-1 arg4-17 ade1 leu2-2*
AGH3	*MAT***a** *met8-1 trp1-1 lys1-1 arg4-17 leu2-2*
D) Mapping strains	
893	*MATα met2-1 lys2*
894	*MAT***a** *met2-1 lys2*
A298-61D	*MATα leu2-3 pet2 arg4 ade8 aro1 trp4 rna3*
A298-65C	*MAT***a** *leu2-3 pet2 arg4 ade8 aro1 trp4 rna3*
CSH-85L	*MATα spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*
CSH-87L	*MAT***a** *spo11 ura3 his2 leu1 lys1 met4 pet8*
CSH-89L	*MAT***a** *spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*
1412	*MAT***a** *tyr1 lys2 ura1 ade2 prt2-2*
A256-99A	*MAT***a** *leu2-3 trp5 lys9 met10 ade1 pet9*
A236-57B	*MAT***a** *leu2-3 trp1 met4 aro7 his3 lys11 suc2 MAL3*
A193-16C	*MAT***a** *leu2-3 met13 ade2 cdc4 pet3 ura4 his4-15 lys2*
A121-3A	*MAT***a** *leu2-3 met14 ade5 pet8 ura3 his7 lys1*
LR663-1D	*MAT***a** *leu2 ura3-52 cdc35-10*
M21-19A	*MAT***a** *met3 trp5 his1*
M21-19B	*MAT***a** *met3 trp5 his1*
E) Other strains	
1327	*MAT***a** *prt3*
1554	*MAT***a** *leu2-3 arg8 pho2 petx prt1*
1555	*MATα leu2-3 arg8 pho2 petx prt1*
9436-3B	*MAT***a** *his4-416R6 ura3-52 ino1-13 gcd1-1*
9436-10D	*MATα his4-416R6 ino1-13 gcd1-1*

tween the *HIS4* proximal region, containing the initiation mutation, and the *lacZ* gene of *Escherichia coli* (DONAHUE *et al.* 1988). Thus, an external suppressor mutation should give rise to His$^+$ colonies that have a blue phenotype on X-gal indicator plates.

We describe here the genetic characterization of three genes, *SUI1*, *SUI2* and *sui3*, that, when mutated, allow initiation events to occur at non-AUG codons. *sui1* and *sui2* are recessive suppressors that also confer a temperature-sensitive phenotype for growth, and were mapped to chromosomes *XIV* and *X*, respectively. Suppression is specific for initiator codon mutations and is the result of increased levels of the protein products expressed from *HIS4* messages that

lack the AUG start codon. *sui2* and *SUI3* suppressor mutations also cause an increase in *HIS4* transcription mediated by the general amino acid control system of yeast.

MATERIALS AND METHODS

Yeast strains, media and genetic methods: The genotypes of strains utilized in this analysis are described in Table 1. All strains used in the reversion analysis are related to TD28, an ascospore derivative of yeast strain S288C (*MATα*) that has been used extensively for the characterization of *HIS4* transcription (DONAHUE, FARABAUGH and FINK 1982; DONAHUE *et al.* 1983; NAGAWA and FINK 1985) and translation initiation (DONAHUE and CIGAN 1988; CIGAN, PABICH and DONAHUE 1988; DONAHUE *et al.* 1988).

The construction of strains containing initiator codon mutations at the *HIS4* locus has been described (DONAHUE and CIGAN 1988), as was the construction of strains containing two copies of the initiator mutations, one at the *HIS4* locus, and one as a proximal *HIS4-lacZ* fusion that is integrated at the *URA3* locus as part of a YIp5 vector (DONAHUE *et al.* 1988).

Standard genetic methods and media have been previously described (SHERMAN, FINK and LAWRENCE 1972). Genetic linkage data and map distances were based on MORTIMER and SCHILD (1985).

Selection of revertants: Spontaneous revertants of the initiator codon mutant strains were selected by demanding growth on synthetic dextrose (SD) medium lacking histidine. Strains were grown to confluence at 23° on YEPD (yeast extract, peptone, dextrose) plates, replica plated onto SD minus histidine plates, and incubated for approximately one week at 23°. His$^+$ papillae were streaked on YEPD plates and retested for their His$^+$ phenotype and for *HIS4-lacZ* expression on SD complete plates containing the X-gal indicator, 5-bromo-4-chloro-3-indolyl-β-D-galactoside (CASADABAN *et al.* 1983). His$^+$ colonies that were blue on X-gal plates within 1–3 days of incubation were further analyzed.

β-Galactosidase assays: Overnight cultures grown at 23° in SD liquid medium supplemented with histidine, leucine, uracil and inositol were diluted in 10 ml of the same medium to an OD_{600} of 0.1 and then grown at 23° to an OD_{600} of 1.2. The cells were harvested and cell extracts prepared by a modification of ROSE, CASADABAN and BOTSTEIN (1981), as described in DONAHUE and CIGAN (1988). β-Galactosidase specific activity was measured from the extracts as described in MILLER (1972). Total protein concentration was determined by the dye-binding method of Bradford (Bio-Rad Laboratories). Standard protein curves were performed with bovine serum albumin. The specific activity in cell extracts was determined from three independent sets of experiments.

Northern analysis: Total RNA was prepared as described (CARLSON and BOTSTEIN 1982) from cells grown to an OD_{600} of 1.2 on SD liquid medium supplemented with uracil, leucine, inositol and histidine. The RNA preparation was enriched for poly(A$^+$) RNA by chromatography on an oligo-dT cellulose column (Collaborative Research). Poly(A$^+$) RNA (20 μg) was subjected to electrophoresis on a 1% denaturing agarose gel (THOMAS 1980) and transferred to GeneScreen membranes (New England Nuclear). Pre-hybridization and hybridization conditions were as recommended by the membrane supplier. The filter was first probed with a nick-translated [α-^{32}P]dATP labeled *HIS4 SalI* fragment corresponding to 536 nucleotides of the 5′ end of the transcript and then exposed to X-ray film. After exposure the probe was removed by boiling the filter and the filter was rehybridized to ^{32}P-labeled YRp7 plasmid DNA for detection of the *TRP1* transcript, which served as an internal control for relative levels of *HIS4* mRNA, as *TRP1* mRNA is not subject to derepression by the general amino acid control. The two films were then superimposed and subsequently photographed to arrive at Figure 3.

Immunoprecipitation of His4-β-galactosidase fusion protein: Wild type and *sui1*, *sui2* and *SUI3* suppressor strains were grown on YEPD medium to an OD_{600} of 1.0 and cell extracts prepared as previously described (DONAHUE and CIGAN 1988). Immunoprecipitations were performed as described (CIGAN *et al.* 1989), using anti-β-galactosidase antiserum (Cappel) at a 1/200 dilution. Complete immunoprecipitation of the fusion protein was determined by β-galactosidase assays of supernatants after Staphylococcus A cell precipitation of the immune complex. Immunoprecipitates were visualized on 7% SDS-polyacrylamide gels by silver staining.

RESULTS

Isolation of external suppressors of initiator codon mutations: Spontaneous reversion analysis was performed with haploid yeast strains containing the mutant initiator codons CUG, GUG, ACG, AUU and ACC, that were present both at the *HIS4* locus and at the *HIS4-lacZ* reporter construction that was integrated at the *URA3* locus (DONAHUE *et al.* 1988). His$^+$ revertants that formed blue colonies on X-gal plates were identified for each initiator codon mutant strain. Greater than 80% of the His$^+$ revertants formed blue colonies on X-gal plates, suggesting that the majority of the mutational events leading to restoration of *HIS4* expression occurred at loci other than *HIS4*.

An initial phenotypic characterization of these revertants revealed two general classes; revertants that were His$^+$, blue (on X-gal), and temperature-sensitive (Ts$^-$) for growth on enriched medium (YEPD) at 37°, and His$^+$, blue revertants that grew at 37° (Ts$^+$).

Genetic analysis of temperature-sensitive revertants: His$^+$ revertants that were Ts$^-$ for growth on enriched medium were tested for the dominance or recessiveness of the mutations. Diploids constructed by mating these revertants to His$^-$ strains containing either a deletion at the *HIS4* locus, or the homologous initiator mutation, were unable to grow in the absence of histidine, and showed no growth inhibition at 37°, indicating that the mutation(s) for both the Ts$^-$ and suppressor phenotypes were recessive.

In order to estimate the number of genetic loci represented among the Ts$^-$ revertants, complementation tests were performed. Suppressor strains of opposite mating type were mated by cross-streaking on YEPD plates at 23°, and diploid intersections were analyzed for growth at the restrictive temperature. According to this analysis, two major groups were detected (Table 2). Group I was composed of both noncomplementing and weakly complementing mutants, suggestive of a complex genetic locus. Group II

TABLE 2

Classification of suppressor strains

	His$^+$, blue, Ts$^-$		His$^+$, blue, Ts$^+$
Initiator codon mutations	Group I (*sui1*)	Group II (*sui2*)	Group III (*SUI3*)
AUU	117-8AR4 (*sui1-1*)	117-8AR20 (*sui2-1*)	117-1AR7 (*SUI3-3*)
	117-8AR15 (*sui1-2*)		117-1AR8 (*SUI3-4*)
	117-9AR19b (*sui1-3*)		117-8AR9 (*SUI3-5*)
	118-2CR3 (*sui1-4*)		117-8AR10 (*SUI3-6*)
	118-2CR13 (*sui1-5*)		117-8AR12 (*SUI3-7*)
	JJ6-R3 (*sui1-6*)		117-9AR8 (*SUI3-8*)
	JJ6-R17 (*sui1-7*)		117-9AR12 (*SUI3-9*)
			117-9AR16 (*SUI3-10*)
			118-2CR6 (*SUI3-11*)
			118-2CR11 (*SUI3-12*)
			118-2CR12 (*SUI3-13*)
			118-2CR16 (*SUI3-14*)
			118-2CR20 (*SUI3-15*)
ACG	114-4AR9b (*sui1-8*)	114-7CR11 (*sui2-3*)	114-1DR6 (*SUI3-2*)
	114-4AR20 (*sui1-9*)	123-4AR31 (*sui2-2*)	114-1DR8 (*SUI3-16*)
	114-4AR27b (*sui1-10*)	123-4AR32 (*sui2-4*)	114-7CR1 (*SUI3-17*)
	114-7BR10b (*sui1-11*)	123-4AR40 (*sui2-5*)	114-7CR6 (*SUI3-18*)
	123-3AR24 (*sui1-12*)		123-3BR7 (*SUI3-19*)
	123-3BR12 (*sui1-13*)		123-3BR10 (*SUI3-20*)
	123-4AR4 (*sui1-14*)		123-4AR6 (*SUI3-21*)
	123-4AR10 (*sui1-15*)		123-4AR25 (*SUI3-22*)
	123-4AR29 (*sui1-16*)		
	JJ2-R14 (*sui1-17*)		
CUG	115-8CR12 (*sui1-18*)	115-10CR33 (*sui2-6*)	115-8CR11 (*SUI3-23*)
	115-10CR21 (*sui1-19*)	115-10CR38 (*sui2-7*)	115-10CR20 (*SUI3-24*)
	116-1CR11 (*sui1-20*)	116-3CR18b (*sui2-8*)	115-10CR29 (*SUI3-25*)
			116-1CR6 (*SUI3-26*)
			116-6BR2 (*SUI3-27*)
			116-6BR5 (*SUI3-28*)
			116-6BR9 (*SUI3-29*)
			116-6BR14 (*SUI3-30*)
ACC	113-2AR1 (*sui1-21*)		113-2AR9 (*SUI3-31*)
	113-2AR2 (*sui1-22*)		113-2AR10 (*SUI3-32*)
	113-2AR6 (*sui1-23*)		113-2AR20 (*SUI3-33*)
	113-2AR15 (*sui1-24*)		113-2AR27 (*SUI3-34*)
			113-2BR1 (*SUI3-1*)
GUG			125-5BR4 (*SUI3-35*)

mutants did not show complementation with each other, but complemented all group I mutants to wild type levels. This assignment is consistent with the suppressor phenotypes of these diploids. Ts$^+$ diploids derived from crosses of group I and group II mutant strains were all His$^-$, whereas Ts$^-$ diploids derived from crosses within each group were His$^+$. Given the recessive character of the suppressor mutations, only diploids containing mutations in the same suppressor locus should show the suppressor phenotype.

Suppressor strains were crossed to strains carrying a deletion at the *HIS4* locus, and the meiotic products analyzed for the segregation of the His$^+$ and Ts$^-$ phenotypes. As shown in Table 3, all His$^+$, blue revertants characterized showed the segregation patterns for the His$^+$ phenotype (2^+:2^-, 0^+:4^-, 1^+:3^-) expected for an allele specific suppressor mutation that is unlinked to *HIS4*. In contrast, crosses between the suppressor strains and strains carrying the homologous initiation mutation at the *HIS4* locus generated only the 2His$^+$:2His$^-$ segregation expected for a single nuclear suppressor mutation (Table 4). In these two analyses, the Ts$^-$ phenotype always segregated in a Mendelian fashion (2Ts$^+$:2Ts$^-$) and Ts$^-$ cosegregated with the His$^+$ phenotype (data not shown). This illustrates that the mutations that confer suppression also confer the Ts$^-$ phenotype.

Evidence that the two complementation groups represent two independent loci was obtained by tetrad analysis from crosses between group I and group II suppressor strains. As shown in Table 5, all intragroup crosses generated only His$^+$ meiotic products. In contrast, intergroup crosses generated His$^-$ spores, indicative of recombination between two unlinked sup-

TABLE 3

Tetrad analysis of His⁺, blue revertants crossed to *HIS4* deletion strains

Revertants	Segregation of His⁺ phenotype in tetrads[a]					
	3 spores			4 spores		
	$2^+:1^-$	$1^+:2^-$	$0^+:3^-$	$2^+:2^-$	$1^+:3^-$	$0^+:4^-$
Group I (*sui1*)						
117-8AR4 (*sui1-1*)	0	0	0	3	4	1
118-2CR3 (*sui1-4*)	1	2	0	1	3	1
114-4AR20 (*sui1-9*)	2	0	1	0	1	1
123-3BR12 (*sui1-13*)	0	0	0	2	5	2
123-4AR29 (*sui1-16*)	0	1	1	0	4	1
115-8CR12 (*sui1-19*)	2	0	0	0	3	2
115-10CR21 (*sui1-20*)	0	0	0	6	12	3
Group II (*sui2*)						
117-8AR20 (*sui2-1*)	1	1	2	3	5	5
114-7CR11 (*sui2-3*)	0	0	5	1	0	3
115-10CR33 (*sui2-6*)	0	0	1	1	4	5
Group III (*SUI3*)						
115-8CR11 (*SUI3-3*)	1	1	1	3	18	6
117-1AR7 (*SUI3-3*)	0	7	5	3	5	2
117-1AR8 (*SUI3-4*)	0	0	1	3	1	1
117-8AR9 (*SUI3-5*)	0	1	1	2	2	1
117-8AR10 (*SUI3-6*)	0	1	2	2	2	2
117-8AR12 (*SUI3-7*)	2	4	0	1	0	2
117-9AR8 (*SUI3-8*)	0	2	1	0	3	0
118-2CR6 (*SUI3-11*)	0	1	0	2	6	2
118-2CR11 (*SUI3-12*)	0	2	1	0	2	3
118-2CR12 (*SUI3-13*)	1	1	0	2	3	2
118-2CR16 (*SUI3-14*)	0	0	0	1	5	1
118-2CR20 (*SUI3-15*)	0	2	1	1	3	1
114-1DR6 (*SUI3-2*)	0	2	0	0	5	2
114-1DR8 (*SUI3-16*)	0	1	0	0	4	1
114-7CR1 (*SUI3-17*)	0	10	2	2	2	1
114-7CR6 (*SUI3-18*)	0	2	1	0	4	2
123-3BR7 (*SUI3-19*)	0	1	0	0	8	2
123-3BR10 (*SUI3-20*)	0	0	1	0	8	1
123-4AR25 (*SUI3-22*)	0	8	1	3	23	5
115-10CR20 (*SUI3-24*)	1	0	0	1	2	0
115-10CR29 (*SUI3-25*)	2	4	0	0	0	2
116-1CR6 (*SUI3-26*)	0	0	0	3	5	2
116-6BR2 (*SUI3-27*)	0	1	0	2	5	1
116-6BR5 (*SUI3-28*)	0	0	0	2	7	1
116-6BR9 (*SUI3-29*)	0	1	0	1	7	2
116-6BR14 (*SUI3-30*)	0	0	1	2	6	1
125-5BR4 (*SUI3-35*)	0	1	0	2	6	2
113-2AR9 (*SUI3-31*)	1	1	0	2	3	0
113-2AR10 (*SUI3-32*)	0	0	0	1	1	1
113-2AR20 (*SUI3-33*)	0	2	2	0	4	0
113-2AR27 (*SUI3-34*)	1	0	1	2	5	1
113-2BR1 (*SUI3-1*)	0	1	0	5	3	0

[a] The strain containing the *HIS4* deletion allele *his4-401* used in the different crosses was either TD77, 44-1B, 44-5A, 44-22B, 45-3B, 45-11D or 65-18B.

pressor mutations. Mutations in group I are designated *sui1*, those in group II, *sui2* (*su*ppressors of *i*nitiator codon mutations). Although only a few representatives of each group were analyzed by genetic crosses, all group I suppressors appeared to correspond to *sui1* and group II suppressors to *sui2*, based on their complementation behavior.

Genetic mapping of *sui1*: The fact that *sui1* and *sui2* suppressors were defective for growth at 37° and that they may be implicated in protein synthesis prompted us to cross these strains to previously identified ts mutants that are conditionally defective for protein synthesis. One of these mutations, *prt2* (HARTWELL and MCLAUGHLIN 1968), is located on the left

TABLE 4

Tetrad analysis of His⁺, blue revertants crossed to strains containing the homologous initiator codon mutation at *HIS4*

Crosses[a]	Segregation of His⁺ phenotype in tetrads			
	3⁺:1⁻	2⁺:2⁻	1⁺:3⁻	0⁺:4⁻
Group I				
117-8AR4 (*sui1-1*)	0	17	0	0
118-2CR3 (*sui1-4*)	0	7	0	0
137-4D (*sui1-6*)	0	7	0	0
139-3C (*sui1-7*)	0	19	0	0
123-3BR12 (*sui1-13*)	0	8	0	0
115-10CR21 (*sui1-20*)	0	7	0	0
Group II				
117-8AR20 (*sui2-1*)	0	8	0	0
114-7CR11 (*sui2-3*)	0	8	0	1
115-10CR33 (*sui2-6*)	0	10	0	0
Group III				
117-1AR7 (*SUI3-3*)	0	17	0	0
118-2CR16 (*SUI3-14*)	0	10	0	0
114-7CR1 (*SUI3-17*)	0	7	1	0
115-8CR11 (*SUI3-23*)	0	16	0	0
115-10CR20 (*SUI3-24*)	0	8	0	0
113-2BR1 (*SUI3-1*)	1	9	0	0

[a] The strain containing the homologous initiator codon mutation at *HIS4* used for the cross with a suppressor strain was one of the following: 234-7B, 75-6D, 73-2C, 73-3B, 73-16D, 76-3D, 76-8D, 77-1C, 77-1A, 76-2B and 71-1B. Suppressor strains 137-4D (*MATa his4-303 leu2-3, -112 ino1-13*, His⁺) and 139-3C (*MATα his4-303 ura3-52*, His⁺) are ascospore derivatives of JJ6-R3 and JJ-6R17, respectively.

arm of chromosome *XIV*, tightly linked to *pet2*, which is linked but proximal to the *met2* locus on *XIVL* (MORTIMER and SCHILD 1985). When *sui1* strains were crossed with strains containing these markers, we noted that the Ts⁻ phenotype associated with the *sui1* suppressor locus exhibited tight linkage to *prt2* and *pet2* (Table 6) and was approximately 34.6 cM from the *met2* locus. In addition, we established by genetic analysis that the *prt2*, *pet2* and *met2* markers we used showed the predicted genetic linkage (Table 6) established by previous studies (MORTIMER and HAWTHORNE 1973). Thus we conclude that *sui1* is located on chromosome *XIV*. Genetically, it was not possible to separate *sui1* and *prt2* by tetrad analysis, suggesting perhaps that they represent mutations in the same locus. However, we do not believe that they represent alleles of the same gene, as *sui1* mutants complement the Ts⁻ phenotype of the *prt2* mutant to wild-type levels, and the cloned wild-type *SUI1* gene does not complement the Ts⁻ phenotype of the *prt2* mutant (T. F. DONAHUE, unpublished observations).

Genetic mapping of *sui2*: Because the initial genetic analysis indicated that *sui2* mutations were centromere-linked, *sui2* suppressor strains were crossed to a series of strains containing centromere-linked markers. *sui2* showed linkage to *met3* (Table 7) in several crosses. To verify this location, we crossed a *sui2* strain to a *cdc35* mutant strain, which is tightly linked to the centromere of chromosome *X* (MATSUMOTO *et al.* 1982). As shown in Table 7, this yielded only the parental ditype class segregation pattern (0Ts⁺:4Ts⁻), confirming the localization of *sui2* to the centromere of chromosome *X*. However, we have not been able to assign *sui2* to an arm.

Genetic analysis of His⁺, Ts⁺ revertants: In contrast to the recessive nature of the *sui1* and *sui2* suppressor phenotypes, the majority of His⁺ revertants that were Ts⁺ showed a dominant suppressor phenotype in complementation tests to wild-type strains that contained either a deletion or the homologous initiation mutation at *HIS4* (data not shown). Tetrad analysis of a number of these diploids showed that each dominant suppressor mutation was unlinked to *HIS4* (Table 3) and segregated as a single mutation (Table 4). Seven dominant suppressor strains representing five independent revertants were randomly chosen for crosses to each other to establish their allelic nature. As shown in Table 5, the meiotic products of each cross were all His⁺, indicating that the suppressor mutations are all linked. In contrast, tetrad analysis of crosses between representatives of these suppressor strains and either *sui1* or *sui2* suppressor strains gave rise to His⁻ meiotic products (Table 5). This is consistent with the dominant Ts⁺ suppressors representing mutations at a third gene, *sui3*, which is unlinked to *SUI1* and *SUI2*. However, we have not ruled out the possibility that genetic loci other than *sui3* may be represented in this class of dominant suppressors.

Suppression specificity: During the course of our genetic characterization of *sui1*, *sui2* and *SUI3* suppressor strains, it was apparent that these suppressor mutations could not restore a His⁺ phenotype when a deletion was present at *HIS4*, but could restore His⁺ phenotypes to strains that contained different initiator codon mutations at *HIS4*. Because initiator codon specificity may be important to mechanistic interpretations of suppression, we crossed representative His⁻, *sui1-6*, *sui2-6* and *SUI3-23* strains that contained a deletion at *HIS4* (*his4-401*), to His⁻ strains that contained one of the seven different point mutations at the initiator codon, or a two base change (AUG-ACC), or a precise 3-bp deletion of the AUG, and analyzed the meiotic products for the spectrum of suppression of these *HIS4* alleles. As shown in Figure 1, all three suppressor loci can restore *HIS4* expression when each of the nine changes are present at *HIS4*. Similar studies with His⁻ yeast strains containing the initiator codon mutations AGG and AAG at *HIS4* indicated that *sui1*, *sui2* and *SUI3* suppressors could restore His⁺ growth to these mutant strains (data not shown). Thus all nine possible point mutations at the AUG start codon at *HIS4* could be suppressed. Although this result suggested that the mechanism of suppression could be unrelated to initiation, these *sui1*, *sui2* and

TABLE 5

Independent segregation of suppressor loci

Crosses			His phenotypes in tetrads					
			3 spores			4 spores		
			3⁺:0⁻	2⁺:1⁻	1⁺:2⁻	4⁺:0⁻	3⁺:1⁻	2⁺:2⁻
Group I		Group I[a]						
138-7B (*sui1-6*)	×	114-4AR20 (*sui1-9*)	8					
2069-6A (*sui1-4*)	×	301-3A (*sui1-1*)	2					
2070-3A (*sui1-6*)	×	301-3A (*sui1-1*)	11					
138-7B (*sui1-6*)	×	123-4AR29 (*sui1-16*)				2		
137-4D (*sui1-6*)	×	123-4AR29 (*sui1-16*)	1					
Group II		Group II						
2114-1D (*sui2-2*)	×	2071-2A (*sui2-1*)				4		
2082-7D (*sui2-6*)	×	2071-2A (*sui2-1*)	1			7		
Group III		Group III						
117-1AR7 (*SUI3-3*)	×	115-8CR11 (*SUI3-23*)	8			8		
117-1AR7 (*SUI3-3*)	×	114-7CR1 (*SUI3-17*)	5			1		
2091-11B (*SUI3-23*)	×	113-2BR1 (*SUI3-1*)				20		
115-10CR20 (*SUI3-24*)	×	114-7CR1 (*SUI3-17*)	3			4		
115-10CR20 (*SUI3-24*)	×	117-8AR12 (*SUI3-7*)	4			14		
115-10CR29 (*SUI3-25*)	×	115-8CR11 (*SUI3-23*)	12			4		
Group I		Group II						
118-2CR3 (*sui1-4*)	×	2071-7A (*sui2-1*)	6	2	3			
138-7B (*sui1-6*)	×	114-7CR11 (*sui2-3*)	1	13	1	1		
2070-3D (*sui1-6*)	×	2071-7A (*sui2-1*)		13		4	1	
Group I		Group III						
138-7B (*sui1-6*)	×	115-8CR1 (*SUI3-23*)		14		1		
137-4D (*sui1-6*)	×	115-8CR1 (*SUI3-23*)	1	6	3	1		
Group II		Group III						
115-10CR33 (*sui2-6*)	×	113-2BR1 (*SUI3-1*)		12		2		
115-10CR33 (*sui2-6*)	×	115-8CR11 (*SUI3-23*)		13		2		

Suppressor derivatives used:

118-2CR3: 2069-6A (*MATα his4-303 ura3-52 leu2-3, -112*)
117-8AR4: 301-3A (*MATα his4-303*)
JJ6-R3: 2070-3D (*MATα his4-303 leu2-3, -112*)
2070-3A (*MAT***a** *his4-303 ura3-52 ino1-13*)
117-8AR20: 2071-7A (*MAT***a** *his4-303 ura3-52*)
2071-2A (*MATα his4-303 ura3-52*)
123-4AR31: 2114-1D (*MAT***a** *his4-301 leu2-3*)
2114-8A (*MATα his4-301 ura3-52*)
115-8CR11: 2091-11B (*MAT***a** *his4-300 leu2-3, -112*)
113-2BR1: 272-8B (*MATα his4-300 ura3-1, -3*)
113-2AR9: 291-3A (*MATα his4-300 ura3-52*)
JJ6-R3: 138-7B (*MAT***a** *his4-301 ura3-52*)
137-4D (*MAT***a** *his4-303 leu2-3, -112 ino1-13*)
115-10CR33: 2082-7D (*MAT***a** *his4-302 leu2-3, -112*)

[a] *sui1* × *sui1* mutants experience either poor sporulation and/or germination and hence the small number of 4-spore tetrads analyzed.

TABLE 6

Genetic mapping of *sui1*

Markers	Crosses	Segregation ratios PD	NPD	TT	Map distance (cM)[a]
sui1 × *prt2*	117-8AR4 × 1412	35	0	0	0
	118-2CR3 × 1412	14	0	0	0
sui1 × *pet2*	117-8AR4 × A298-65C	16	0	0	0
sui1 × *met2*	117-8AR4 × 894	41	2	51	33.5
	138-7B × 893	20	1	34	36.4
prt2 × *pet2*	1412 × A298-61D	15	0	0	0
prt2 × *met2*	1412 × 893	14	0	5	13.2
met2 × *pet2*	894 × A298-61D	12	1	11	35.4
	893 × A298-65C	5	0	6	27.3

[a] Map distances were calculated using the equation derived by PERKINS (1949): 100/2 × [(T + 6NPD)/(PD + NPD + T)].

TABLE 7

Genetic mapping of *sui2*

Crosses			Segregation PD	NPD	TT	Map distance (cM)
sui2		*met3*				
117-8AR20	×	CSH-89L	22	0	8	
117-8AR20	×	M21-19B	15	0	2	
2082-8A	×	M21-19A	11	0	2	
			58	0	12 (Total)	10.3
sui2		*cdc35*				
794-1C	×	LR663-1D	22	0	0	0

The genotypes of *sui2* strains are:

2082-8A *MAT***a** *his4-302 sui2-6*, derivative of 115-10CR33

794-1C *MATα his4-306 ura3-52 ino1-13 sui2-1*, derivative of 117-8AR20

SUI3 suppressor strains do not restore expression to known missense, frameshift or nonsense mutations. This was determined by tetrad analysis from crosses with yeast strains AGH1, AGH3, AH2, 5470-1C and TD5 and our genetic observations during the course of mapping the *sui1* and *sui2* genes. That is, all genetic markers in our mapping strains segregated $2^+:2^-$. Thus these results suggest that the *sui1*, *sui2* and *SUI3* strains are specific for suppression of initiator codon mutations and may utilize a site at *HIS4* that lies outside the normal initiator region.

Immunoprecipitation of His4-β-galactosidase produced as a result of suppression: A simple interpretation of our genetic reversion study is that *sui1*, *sui2* and *SUI3* represent mutations in components of the translation initiation complex that restore protein synthesis to the mutant *HIS4* message. Therefore, one prediction is that suppression should lead to an increase in *HIS4* translated product as compared to the isogenic parent strain. To test this we used anti-β-galactosidase antibodies to immunoprecipitate the His4-β-galactosidase gene product synthesized in these strains as a result of either *sui1*, *sui2* or *SUI3* suppression when an AUU codon was present at the normal initiator region in the *HIS4-lacZ* construction. As shown in Figure 2, *sui1*, *sui2* and *SUI3* strains synthesize significant amounts of His4-β-galactosidase that is consistent in M_r value to the His4-β-galactosidase product synthesized by a yeast strain that contains an identical *HIS4-lacZ* fusion construction but with an AUG codon at the normal initiator region. These levels are significantly higher than the His4-β-galactosidase protein present in each isogenic parent strain. However, the levels of translated products made as a result of suppression events differ for the three suppressors, with *SUI3* suppression being most efficient in restoring translation to *HIS4* in the absence of an AUG.

Efficiency of suppression: The relative efficiency of suppression events at *HIS4* was determined by assaying β-galactosidase activities generated from mutant and wild type *HIS4-lacZ* fusion strains. As shown in Table 8, when an AUU is at the initiator site in the *HIS4-lacZ* fusion, *sui1*, *sui2* and *SUI3* suppressors cause significant increases in levels of β-galactosidase, with levels 14-, 11- and 47-fold higher, respectively, than residual levels of β-galactosidase activity observed in the parent strain. Similar assays were performed with *sui1*, *sui2* and *SUI3* strains containing an AUG start codon in the *HIS4-lacZ* construction to monitor the effects of these suppressors on normal initiation events at *HIS4*. As shown in Table 8, the *sui1* suppressor strain has the same relative level of β-galactosidase activity as that observed in the wild-type *HIS4-lacZ* strain. In contrast, *sui2* and *SUI3* suppressor strains contain levels three- to fourfold higher than the wild-type control. These values appear not to be the sum of normal and mutant initiation events, for they are greater than the sum. Rather, the levels of β-galactosidase activity expressed from the *HIS4-lacZ* fusion construction with an AUG initiator codon in the *sui2* and *SUI3* suppressor backgrounds approach the β-galactosidase activity obtained from the same fusion construction in a yeast strain containing a *gcd1* mutation that alters the general amino acid control system and causes maximal induction of transcription at *HIS4* (WOLFNER *et al.* 1975). This is consistent with Northern analysis (Figure 3) of $his4^{AUU}$ mRNA produced from suppressor strains, which show that transcriptional levels may be elevated to that observed in a *gcd1* mutant.

DISCUSSION

Here we report the genetic characterization of strains carrying mutations at one of three suppressor loci in yeast, *SUI1*, *SUI2* and *sui3*, which, when mutated, restore translation initiation at *HIS4* despite the absence of the wild-type AUG start codon. The *sui1* and *sui2* loci have been mapped to chromosomes *XIV* and *X*, respectively. Although *sui1* is tightly linked on

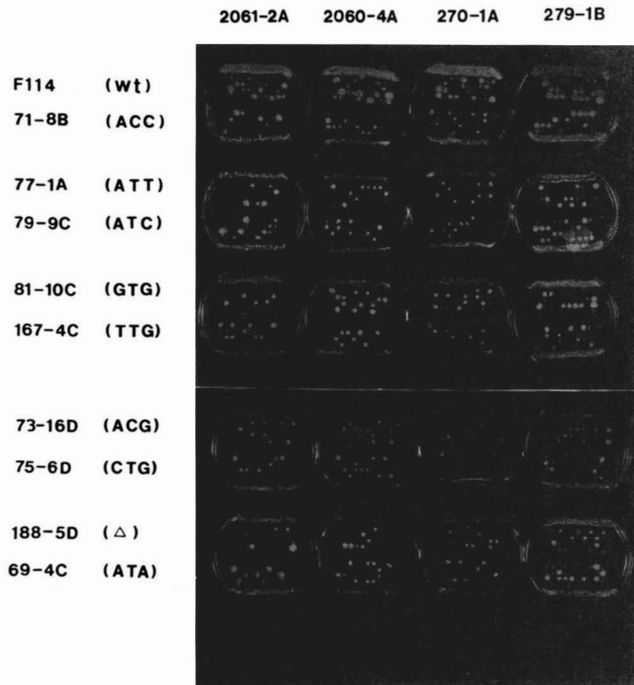

FIGURE 1.—Restoration of *HIS4* expression to different *his4* initiator codon mutant strains by *sui1*, *sui2* and *SUI3* suppressors. The *sui* suppressor strains 2061-2A (*MATa his4-401 ino1-13 ura3-52 sui1-6*, Ts⁻ derivative of JJ2-R14), 2060-4A (*MATa his4-401 ino1-13 ura3-52 sui1-6*, Ts⁻ derivative of JJ6-R3), 270-1A (*MATa his4-401 leu2-3 ura3-52 ino1-13 sui2-6*, Ts⁻ derivative of 115-10CR33) and 279-1B (*MATa his4-401 leu2-3 SUI3-23*, Ts⁺ derivative of 115-8CR11) were crossed to a strain containing a deletion of the initiator codon at *HIS4*, the initiator codon mutant strains and the wild-type control strain F114. Meiotic products were dissected on YEPD plates, grown at 23° and colonies replica plated to SD-His plates and incubated at 23° for 1-3 days.

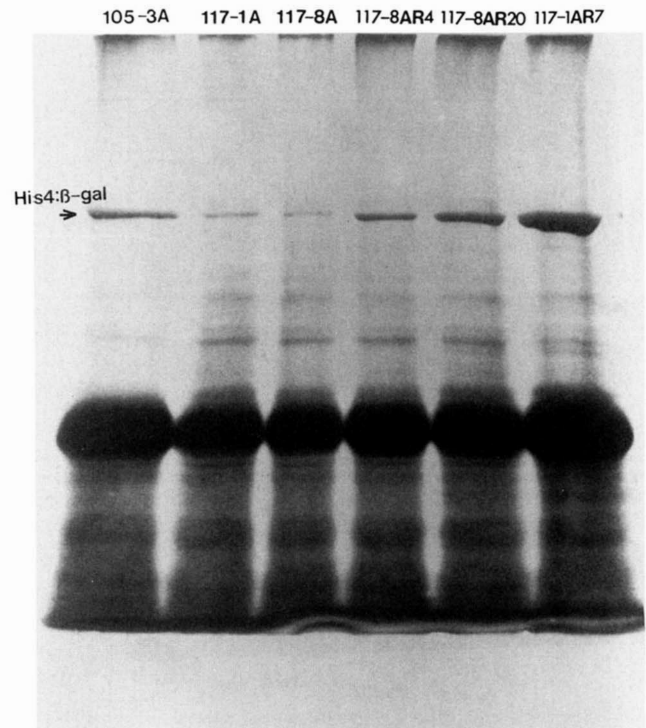

FIGURE 2.—Immunoprecipitation of His4-β-galactosidase fusion products as a result of suppression. Wild-type and *sui1*, *sui2* and *SUI3* suppressor strains were grown on YEPD media to an OD_{600} of 1.0 and cell extracts prepared as previously described (DONAHUE and CIGAN 1988). Immunoprecipitations were performed as described (CIGAN *et al.* 1989), using anti-β-galactosidase antiserum (Cappel) as a 1/200 dilution. The completeness of immunoprecipitation of the fusion protein was determined by β-galactosidase assays of supernatant after Staphylococcus A cell precipitation of the immune complex. Immunoprecipitates were visualized on 7% SDS-polyacrylamide gels by silver staining. Immunoprecipitation was performed with cell extracts (10 mg of total protein) prepared from the suppressor parent strains 117-1A and 117-8A, and from the suppressor strains 117-8AR4 (*sui1-3*), 117-8AR20 (*sui2-1*) and 117-1AR7 (*SUI3-2*). All strains contain a $his4^{AUU}$*-lacZ* fusion construction. The product of a wild-type *HIS4-lacZ* fusion (5 mg) in a wild-type strain 105-3A is included as a control.

chromosome *XIV* to the *prt2* mutation, a mutation that causes a conditional defect in protein synthesis, *SUI1* and *PRT2* appear to be separate and distinct genes based on complementation studies of mutants and the cloned *SUI1* gene. Similarly, *sui2* shows tight linkage to *cdc35*, but the two are different genes, since the DNA sequences (*SUI2*: CIGAN, FENG and DONAHUE 1988; *CDC35*: KATAOKA, BROEK and WIGLER 1985 and MASSON *et al.* 1986) are different. Tentatively, the *SUI3* locus has been assigned to chromosome *XVI* based on chromoblot analysis (B. CASTILHO-VALAVICIUS, F. WINSTON and T. DONAHUE, unpublished observations).

We anticipated that suppressor analysis applied to initiation codon mutants might prove to be an effective method of defining components of the translation initiation complex that interact with mRNA or function in mediating start site selection during the scanning process in yeast, since similar approaches applied to missense, nonsense or frameshift mutations have proven effective in identifying components of the translational elongation complex (MURGOLA 1985; SHERMAN 1982; SANDBAKEN and CULBERTSON 1988). Our basic characterization of *sui1*, *sui2* and *SUI3* suppressor strains is consistent with their having defects in the initiation machinery. Namely, all suppressor mutations impair growth properties to yeast strains on enriched medium, with *sui1* and *sui2* suppressor mutations causing temperature-sensitive growth. All three suppressors are specific for initiator codon mutations and do not suppress known nonsense, frameshift and missense mutations. All suppressors appear to act at the level of translation, resulting in increased levels of protein products as indicated by immunoprecipitation of His4-β-galactosidase fusion protein synthesized in yeast from an initiator codon defective message. That the mechanism of suppression is common and results in an altered initiation start site has been confirmed by purification and amino-terminal sequence analysis of the His4-β-galactosidase products made in the *sui1* (our unpublished observations), *sui2* (CIGAN *et al.* 1989) and *SUI3* (DONAHUE *et al.* 1988)

TABLE 8

Efficiency of suppression

Strain[a]	Hybrid message	Genetic background	β-Galactosidase specific activity	Percent of wild-type value	-Fold increase over initiation mutant
105-3A	*HIS4*^{AUG}*-lacZ*	wt	176.2	100	44
117-8A	*his4*^{AUU}*-lacZ*	wt	4.0	2.3	1
117-8AR4	*his4*^{AUU}*-lacZ*	*sui1*	57.8	32.3	14.4
117-8AR20	*his4*^{AUU}*-lacZ*	*sui2*	43.2	24.5	10.8
117-1AR7	*his4*^{AUU}*-lacZ*	*SUI3*	188.7	107.1	47.2
2227-7A	*his4*^{AUU}*-lacZ*	*gcd1*	20.0	11.3	5.0

Strain[a]	Hybrid message	Genetic background	β-Galactosidase specific activity	-Fold increase over wild-type repressed value
105-3A	*HIS4*^{AUG}*-lacZ*	wt	176.2	1.0
2226-1A	*HIS4*^{AUG}*-lacZ*	*gcd1*	822.0	4.7
2223-8A	*HIS4*^{AUG}*-lacZ*	*sui1*	208.0	1.2
2210-7B	*HIS4*^{AUG}*-lacZ*	*sui2*	543.3	3.1
2224-17C	*HIS4*^{AUG}*-lacZ*	*SUI3*	697.0	4.0

[a] The genotypes of suppressor derivative strains are:

2227-7A *MATα his4-303 gcd1-1 ura3-52::his4*(AUU)*-lacZ* (Ura$^+$)
2226-1A *MAT***a** *his4-303 gcd1-1 ura3-52::HIS4*(AUG)*-lacZ* (Ura$^+$)
2223-8A *MATα his4-303 ura3-52::HIS4*(AUG)*-lacZ* (Ura$^+$) *sui1-3* (derivative of 117-8AR4)
2210-7B *MAT***a** *his4-303 ura3-52::HIS4*(AUG)*-lacZ* (Ura$^+$) *sui2-1* (derivative of 117-8AR20)
2224-17C *MAT***a** *his4-303 ura3-52::HIS4*(AUG)*-lacZ* (Ura$^+$) *SUI3-2* (derivative of 117-1AR7).

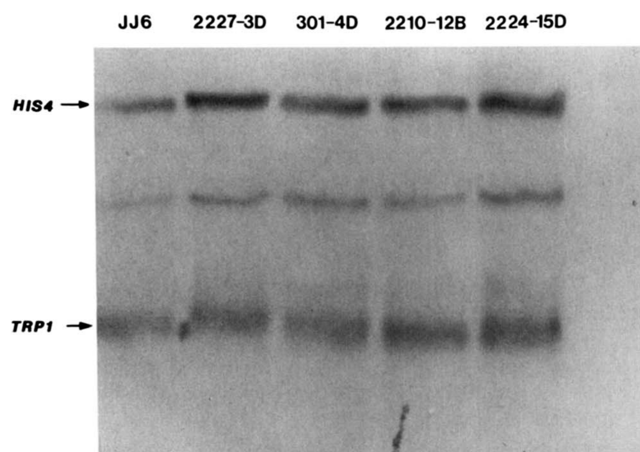

FIGURE 3.—Northern analysis of *his4*^{AUU} mutant message. Poly(A$^+$) RNA was isolated from yeast strains that contained the *his4* AUU initiator codon allele in either a wild type (JJ6), *gcd1-1* (2227-3D: *MATα his4-303 ura3-52 ino1-13 gcd1-1*), *sui1* (301-4D: *MAT***a** *his4-303 ura3-52 leu2-3 sui1-3*), *sui2* (2210-12B: *MATα his4-303 ura3-52 leu2-3, -112 sui2-1*) or *SUI3* (2224-15D: *MATα his4-303 ura3-52 leu2-3, -112 SUI3-2*) genetic backgrounds. Blots were probed with a nick-translated [α-^{32}P]dATP labeled *HIS4 Sal*I fragment corresponding to 536 nucleotides of the 5′ end of the transcript and then exposed to X-ray film. After exposure the probe was removed by boiling the filter and the filter was rehybridized to ^{32}P-labeled YRp7 plasmid DNA for detection of the *TRP1* transcript, which served as an internal control for relative levels of *HIS4* mRNA, as *TRP1* mRNA is not subject to derepression by the general amino acid control system.

suppressor backgrounds. These studies have shown that a UUG codon that is common to the *HIS4* coding region of all of our constructions, located at amino acid position three in the *HIS4* coding region, is used as the start site.

Recent studies from our laboratory on the molecular characterization of these genes have shown that the *sui2* locus encodes the α subunit (CIGAN *et al.* 1989) and *SUI3* the β subunit (DONAHUE *et al.* 1988) of the eukaryotic translation initiation factor, eIF-2, of yeast. Preliminary studies with the cloned *sui1* gene indicate that it also encodes a protein product. The interallelic complementation behavior of some of the *sui1* mutants implies that this gene product may be a multimeric cellular component with complex function.

One interesting aspect of our analysis is the observation that both *sui2* and *SUI3* suppressor mutations may confer derepression of *HIS4* transcription through the general amino acid control of yeast. This suggests that the values for suppression efficiency of *sui2* and *SUI3* observed when AUU is present at the *HIS4-lacZ* fusion may be inflated as a consequence of transcriptional induction of *HIS4* (Table 8). However, this transcriptional induction cannot fully account for restored translational expression in the mutant fusions. The *gcd1* mutation that causes maximal transcription induction at *HIS4* does not confer a His$^+$ phenotype to initiator mutants (data not shown) nor does it increase β-galactosidase levels in a *HIS4-lacZ* fusion strain to the same level as *sui1*, *sui2* or *SUI3* (Table 8). Furthermore, steady state levels of the mutant *HIS4* transcript observed in each suppressor background are not elevated above the levels we ob-

serve in a *gcd1* mutant (Figure 3). Therefore suppression events that lead to increased levels of His4-β-galactosidase and a His^+ phenotype must reflect increases in translated products from the mutant messages that lack an AUG start codon. Correcting for such transcriptional induction suggests that the efficiency of suppression in these *sui1*, *sui2* and *SUI3* strains is approximately 27%, 8% and 27% of wild-type translation, respectively.

One possible explanation for *sui2* and *SUI3* suppressor mutations altering general amino control is that each may result in inefficient translation of mRNAs for aminoacyl synthetases, or amino acid biosynthetic genes, and thus result in an amino acid starvation signal. This signal would then induce the general amino acid control system that results in increases in *HIS4* transcription. However, recent studies suggest that at least one effect of mutant *sui2* and *SUI3* genes is to directly alter the translational regulation of the *GCN4* locus (WILLIAMS, HINNEBUSCH and DONAHUE 1989), which encodes the positive activator of transcription of genes subject to general amino acid control. This regulation is mediated by four out of frame AUG codons in the upstream noncoding region of the *GCN4* mRNA (HINNEBUSCH 1988). Thus, our selection scheme was not only effective in identifying basic components of the translation initiation complex that mediate start site selection but also components that may function in mediating translational regulatory mechanisms in yeast.

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