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Codon Recognition During Frameshift Suppression in *Saccharomyces cerevisiae*†

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A genetic approach has been used to establish the molecular basis of 4-base codon recognition by frameshift suppressor tRNA containing an extra nucleotide in the anticodon. We have isolated all possible base substitution mutations at the position 4 (N) in the 3′-CCCN-5′ anticodon of a *Saccharomyces cerevisiae* frameshift suppressor glycine tRNA encoded by the *SUF16* gene. Base substitutions at +1 frameshift sites in the *his4* gene have also been obtained such that all possible 4-base 5′-GGGN-3′ codons have been identified. By testing for suppression in different strains that collectively represent all 16 possible combinations of position 4 nucleotides, we show that frameshift suppression does not require position 4 base pairing. Nonetheless, position 4 interactions influence the efficiency of suppression. Our results suggest a model in which 4-base translocation of mRNA on the ribosome is directed primarily by the number of nucleotides in the anticodon loop, whereas the resulting efficiency of suppression is dependent on the nature of position 4 nucleotides.

Although mRNA is translated into protein almost exclusively through the use of a triplet genetic code, instances of nontriplet reading resulting in a shift in reading frame have been shown to occur in both procaryotes and eucaryotes (1, 5, 13, 28–30). Shifts from the normal triplet reading frame have been invoked as an explanation to account for such phenomena as the leaky expression of genes containing certain frameshift mutations and the simultaneous expression of out-of-phase overlapping genes (1, 13, 19). The analysis of such nontriplet reading systems may help us understand the molecular basis of low-level frameshifting known to occur in normal cells and the means by which nontriplet reading might serve in specific cases as a translocational regulatory mechanism.

We have examined the role of tRNA in nontriplet reading of the genetic code by analyzing informational suppressors of frameshift mutations in *Saccharomyces cerevisiae*. Normal triplet reading of an altered mRNA containing a single nucleotide insertion (+1 frameshift mutation) usually results in the production of a nonfunctional protein since the mutation causes a shift from the proper reading frame. However, certain +1 frameshift mutations can be suppressed and the proper reading frame can be restored by an appropriately altered tRNA containing an extra nucleotide in the anticodon (8, 14, 31).

Procaryotic frameshift suppressor tRNAs translate specific classes of 4-base mRNA sequences. However, the molecular basis for specificity of codon recognition remains unresolved because different frameshift suppressors exhibit contrasting codon recognition patterns. For example, the product of the *Salmonella* frameshift suppressor gene *sufD*, a mutant $tRNA^{Gly}$ containing a 3′-CCCC-5′ anticodon, can apparently only translate 4-base codons that allow standard base pairing at all four anticodon-codon positions (2, 31). In contrast, the product of the *Salmonella* frameshift suppressor gene *sufJ*, which is thought to encode an altered $tRNA^{Thr}$, is able to recognize the codons 5′-ACCA-3′, 5′-ACCC-3′, and 5′-ACCU-3′ (2). Since it is unlikely that the *sufJ* anticodon contains inosine, this suppressor should not be able to recognize all three codons by standard base pairing or wobble. Although different suppressor tRNAs may be subject to different codon recognition constraints, as suggested by Roth (33), the contrast between *sufD* and *sufJ* represents a paradox that is presently not understood.

Recognition of nontriplet codons by altered tRNA can be examined in yeast cells by analysis of frameshift suppressors and suppressible mutations (5, 7, 9, 14, 15). We have previously shown that the frameshift suppressor gene *SUF16-1* encodes a $tRNA^{Gly}$ containing the mutant anticodon 3′-CCCG-5′ in place of the wild-type 3′-CCG-5′ anticodon (14). *SUF16* is one of 16 loci in the *S. cerevisiae* genome defined by mutations that result in the suppression of +1 frameshifts in glycine codons at the *his4* locus (15). These suppressors have been examined for their ability to suppress the +1 G:C frameshift mutations *his4-38* and *his4-519*, which generate the mutant 4-base glycine codons 5′-GGGU-3′ and 5′-GGGG-3′, respectively (10, 15). Mutations mapping at 6 loci suppress both mutations, whereas those mapping at the remaining 10 loci, including *SUF16-1*, fail to suppress *his4-519*. These observations led us to suspect that differential suppression might reflect nucleotide differences inherent in the anticodons of different glycine tRNA isoacceptors, an assumption now verified experimentally (14; unpublished data, this laboratory). Since these results suggest the existence of specific rules governing tRNA-mediated 4-base codon recognition, we designed a genetic system that would elucidate these rules.

The apparent inability of the *SUF16-1* tRNA to recognize 5′-GGGG-3′ codons allowed us to select alternate alleles of *SUF16* and *his4* that permit successful anticodon-codon interactions leading to a His^+ phenotype. This approach led to the identification of a series of *SUF16* alleles containing all possible 4-base 3′-CCCN-5′ anticodon sequences. In addition, all possible 4-base 5′-GGGN-3′ codons have been identified at *his4*. We show that codon recognition by frameshift suppressor tRNA does not require position 4 base pairing. Nonetheless, position 4 interactions influence the

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efficiency of suppression. The role of position 4 nucleotides in frameshift suppression is discussed.

MATERIALS AND METHODS

Strains, plasmids, and genetic methods. The following *S. cerevisiae* strains were used for the isolation of mutations affecting codon recognition: R453 (*leu2-3 his4-211 ura3-52 suf16*$^+$ [YIPSUF16-1] *MAT*α), R564 (*leu2-3 his4-211 ura3-52 SUF16-4 MAT*α), and R379 (*leu2-3 his4-519 ura3-52 trp1-1 SUF16-1 MAT***a**). Strain R453 contains a duplication of the *SUF16* region generated by integration of a copy of plasmid YIpSUF16-1 (14) at the *SUF16* locus on chromosome III. The mutations *SUF16-2*, *SUF16-3*, *SUF16-4*, and *his4-211R1* were isolated in strain R453. Each of these mutations was subsequently introduced into a haploid strain containing a single copy of the *SUF16* gene by the method of gene replacement (34).

Escherichia coli 6507 (HB101 *hsdR hsdM leu pro recA pyrF74*::Tn*5*) was obtained from D. Botstein and used in bacterial transformation (27) and plasmid DNA preparation (17). Yeast transformation was performed by the method of Hinnen et al. (18) as modified by Gaber and Culbertson (14) or by the method of Ito et al. (21). Yeast DNA was prepared by the method of Olson et al. (26).

Plasmid YIp309 was generously provided by G. R. Fink. This plasmid consists of YIp5 (38) plus an insertion of a 3.5-kilobase *Eco*RI fragment that includes most of the wild-type *HIS4A* region as well as adjacent sequences at the 5′ end of the *HIS4* gene (11). Plasmid YIp309 was used in cloning *his4* alleles as described below.

Genetic methods and nomenclature are described in the Cold Spring Harbor yeast course manual (36). Growth media for bacteria and yeasts are described by Miller (24) and Gaber and Culbertson (15), respectively.

Cloning and sequencing *his4* and *SUF16* alleles. Alleles of the *his4* gene described in this study were cloned by the method of plasmid integration and excision (32) with the following changes. To generate a duplication of the *his4* region, plasmid YIp309 carrying the *his4A Eco*RI fragment was used in place of a plasmid carrying the smaller *Sal*I fragment. Yeast DNA prepared from transformants was digested with *Hin*dIII or *Bam*HI before ligation to clone mutations in *his4A* or *his4B*, respectively. The strategy for sequencing *his4A* mutations has been described by Donahue et al. (10, 11). Mutations in *his4B* were located on a 346-base-pair *Hin*dIII-*Taq*I fragment containing the 5′ proximal *his4B* region.

Alleles of the *SUF16* gene were cloned by a modified version of the plasmid integration and excision procedure described by Gaber and Culbertson (14). Plasmid YIpSUF16-1 carrying the *SUF16-1* allele was used in place of plasmid YIpRG1 to generate a duplication of the *SUF16* region. The cloning procedure and sequencing strategy were otherwise identical to those previously described.

The parental strain R453 contains a heterozygous duplication of the *SUF16* region generated as described above. To clone mutations derived from this strain that mapped at *SUF16*, DNA carrying pBR322 and the mutated copy of *SUF16* was excised by cleavage with *Sal*I, ligated, and recovered by transformation of *E. coli*. To clone mutations mapping at *his4*, it was necessary to first remove the *SUF16* duplication and then generate a duplication of the *his4* region before cloning. Removal of the *SUF16* duplication was accomplished by screening for loss of the Ura$^+$ phenotype conferred by the *URA3* gene on the vector located between the duplicated segments. Loss of the *URA3* phenotype occurs by recombination within the duplication, resulting in deletion of the plasmid and one copy of the duplicated segment (34).

The formation of appropriate duplications required for the cloning of mutations derived from strains R564 and R379 was accomplished after mutant isolation. In all cases, plasmids retrieved in *E. coli* were reintroduced into *S. cerevisiae* by transformation and tested phenotypically to ensure that the correct gene copy and allele had been cloned.

DNA sequences were determined by the method of Maxam and Gilbert (23). The base change associated with each *his4* mutation was deduced by comparing the mutant sequence with that of the published wild-type *HIS4* DNA sequence (11). DNA fragments to be sequenced were 3′ end labeled with avian myeloblastosis virus reverse transcriptase (37) obtained from the Division of Cancer Cause and Prevention, National Cancer Institute. [α-^{32}P]deoxynucleoside triphosphates (810 Ci/mmol) were obtained from Amersham Corp.

Assay for gene conversion in heterozygous duplication strains. Reversion of strain R453 to a His$^+$ phenotype might have resulted from gene conversion of the heterozygous duplication of the *SUF16* gene to a homozygous *SUF16-1* duplication. This possibility was tested for each revertant before molecular analysis by screening among segregants for recombination events that delete the *URA3* gene located within the duplicated segment. If the duplication is heterozygous (*suf16*$^+$/*SUF16-1*), two phenotypic classes of Ura$^-$ segregants (His$^+$ Leu$^+$ or His$^-$ Leu$^-$) are expected, depending on which copy of the *SUF16* gene is deleted. The appearance of Ura$^-$ His$^-$ Leu$^-$ segregants, which occur at an approximate frequency of 10^{-3} to 10^{-4} per cell plated, signals heterozygosity of the duplicated *SUF16* gene.

Temperature-sensitive suppression. Temperature-sensitive suppression of *his4* frameshift mutations was distinguished from temperature-sensitive growth on undefined 1% yeast extract–2% Bacto-Peptone–2% dextrose (YEPD) medium as follows. Strains exhibiting heat-sensitive suppression failed to grow at 37°C on defined medium lacking histidine, whereas such strains grew at 30°C on medium lacking histidine and at 37°C both on defined medium supplemented with histidine and on YEPD medium.

Chromosome III disomy. N+1 disomy for chromosome III was determined in crosses by monitoring meiotic segregation of *SUF16* and *MAT1* (mating type), which along with the *his4* gene are located on chromosome III (15, 25). His$^+$ strains to be tested for disomy were crossed with a *suf16*$^+$ strain, and tetrads were analyzed from the heterozygous diploid. In addition to the observed aberrant segregation of the suppressor expected in tetrads derived from a *SUF16*/*SUF16*/*suf16*$^+$ trisomic diploid, the codominant mating-type alleles resulted in the appearance of tetrads containing nonmating spores according to theoretical expectation for chromosome III disomy in the suppressor-bearing parental strain (6, 35).

Histidinol dehydrogenase activity. Histidinol dehydrogenase activity was assayed by measuring the conversion of ^{14}C-histidinol to ^{14}C-histidine. Radioactive histidinol was isolated from *Salmonella typhimurium* D1 (genotype *hisD1*, obtained from G. R. Fink) by the method of Ciesla et al. (4). Yeast strains to be assayed for enzyme activity were grown to mid-log phase, permeabilized in 40% dimethyl sulfoxide, and standardized by diluting each culture to an absorbance at 550 nm of 1.0. Diluted cells (50 μl) were added to 90 μl of a reaction mixture containing 125 mM Tris-chloride (pH 9.6), 15 mM NAD$^+$, and 10 μl of ^{14}C-histidinol (22,000 cpm). At

30°C, the reaction is linear for more than 1 h. After terminating the reaction, ^{14}C-histidine was purified by Dowex-50 ion-exchange chromatography and counted in Aquasol.

Enzyme activities for strains of genotypes *SUF16-2 his4-211R1* and *SUF16-2 his4-519R1* represent the average activities obtained by assaying six independently constructed strains of each genotype (see Fig. 6). Background enzyme activity was assayed in *his4-211R1* and *his4-519R1* strains lacking a suppressor. The background determined in this way was identical to a no-enzyme control. After subtracting the background, data was analyzed by a *t*-test to locate error bars and to determine statistical significance.

RESULTS

Apparent failure of *SUF16-1* suppressor tRNA to recognize 5′-GGGG-3′ codons. The mRNA sequence changes of two previously analyzed +1 frameshift mutations, *his4-38* (5′-GGU-3′→5′-GGGU-3′) and *his4-519* (5′-GGG-3′→5′-GGGG-3′) (10), provided the initial basis for determining parameters that influence 4-base codon recognition. The suppressor tRNA encoded by *SUF16-1* (anticodon 3′-CCCG-5′) was previously shown to suppress *his4-38* and was therefore presumed to be capable of translating 5′-GGGU-3′ codons (14, 15). However, the apparent lack of suppression of *his4-519* suggested an inability of this tRNA to recognize 5′-GGGG-3′ codons.

As a preliminary test of these assumptions, advantage was taken of the observation that another frameshift mutation, *his4-211*, exhibited a pattern of suppressibility identical to that of *his4-519* (5, 15). A DNA fragment containing the *his4-211* mutation was cloned by the method of plasmid integration and excision (see above), and the mutational change was deduced by comparing the mutant DNA sequence (Fig. 1) with that of the wild type (11). The mutant sequence differs from the wild-type sequence by a single G:C base-pair addition within four consecutive G:C base pairs located in the *his4B* region at position +738 to 741. Thus, the *his4-211* codon sequence 5′-GGGG-3′ is identical to the *his4-519* codon sequence located at position +493 to 495 in the *his4A* region (10). The similarity of these mutations suggested a common basis for an inability to be suppressed by *SUF16-1* and led to further experiments to determine the molecular basis of 4-base codon recognition.

Mutations affecting codon recognition. The following genetic approach was used to isolate mutations affecting codon recognition. We examined His$^+$ revertants of the phenotypically His$^-$ strain R453, which among other markers contains *leu2-3* (a *SUF16-1*-suppressible frameshift mutation), *his4-211* (5′-GGGG-3′), and a *SUF16-1* (3′CCCG-5′)/*suf16*$^+$ (3′-CCG-5′) heterozygous duplication (see above). His$^+$ revertants of this strain, which occur at an approximate frequency of 10^{-8} per cell plated, were shown by genetic analysis to result from four classes of genetic events: (i) mutations at *his4* that confer a His$^+$ phenotype in the absence of *SUF16-1* (true or internal second-site revertants), (ii) suppressor mutations unlinked to *SUF16* or *his4*, (iii) mutations linked to *SUF16-1* that confer suppression of *his4-211*, and (iv) mutations linked to *his4-211* that permit suppression by *SUF16-1*. None of the revertants were aneuploid for chromosome III which carries the *SUF16* and *his4* loci (15, 25). The first two classes were not analyzed further. The last two classes, which were expected to include the mutations of interest, were screened phenotypically by suppression tests to establish any genetic differences within each class. Subsequently, DNA fragments carrying the mutations were cloned and examined by DNA sequence analysis.

Nucleotide substitutions in the *SUF16* tRNA. His$^+$ revertants that contained a *SUF16-1*-linked mutation were first examined to determine whether reversion resulted from gene conversion of the heterozygous duplication present in the parent strain, since it was possible that two copies of the *SUF16-1* allele might confer suppression of *his4-211* (see above). None of the representative revertants examined were phenotypically His$^+$ as the result of gene conversion. Subsequently, these revertants were examined for possible differences in suppression of the *leu2-3* and *his4-211* frameshift mutations. The suppression tests revealed three phenotypic subclasses of mutants, all of which were Leu$^+$ His$^+$ at 30°C but which could be readily distinguished at 37°C. Mutants of subclass 1 were Leu$^+$ His$^+$ at 37°C, and mutants of subclass 2 were Leu$^+$ His$^-$ at 37°C. For reasons to be discussed in a subsequent section, the Leu$^+$ phenotype of subclass 2 mutants was very weak but distinguishable from a Leu$^-$ phenotype. Mutants of subclass 3 were Leu$^-$ His$^-$ at 37°C.

DNA fragments containing the *SUF16* suppressor region were cloned from representatives of each of the three phenotypic subclasses and sequenced (see above). The results demonstrate that these mutations correspond to single-base-pair substitutions at position 4 in the anticodon of the suppressor tRNA (Fig. 2 and 3). Subclasses 1, 2, and 3 contain new alleles, designated *SUF16-2*, *SUF16-3*, and *SUF16-4*, that encode tRNA products containing the 4-base anticodons 3′-CCCC-5′, 3′-CCCA-5′, and 3′-CCCU-5′, respectively.

These results suggest that the 3′-CCCG-5′ anticodon of the *SUF16-1* tRNA cannot efficiently recognize the *his4-211* 5′-GGGG-3′ codon. However, substitution of G with C, A, or U at position 4 in the tRNA appears to enhance recognition of this codon. To confirm this conclusion, we have also shown that these base substitutions allow recognition of the *his4-519* 5′-GGGG-3′ codon. Taken collectively, these results suggest that position 4 base combinations that are compatible with suppression include a C/G base pair, an A/G nonstandard pair, and a U/G pair with potential for wobble. These and subsequent position 4 designations conform to the

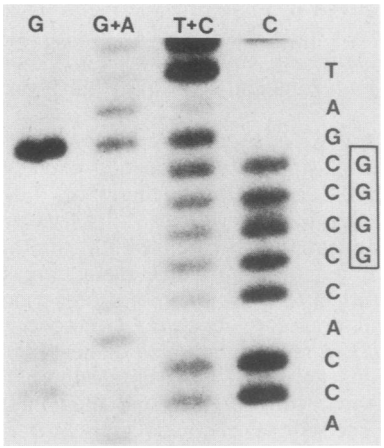

FIG. 1. DNA sequence of the *his4-211* mutation. The DNA sequence shown was compared with the corresponding wild-type sequence of the *HIS4B* region determined by Donahue et al. (11). The results indicate that the *his4-211* mutation is a +1 G:C insertion within four consecutive G:C base pairs at position +738 to +741. The location of the predicted 4-base codon 5′-GGGG-3′ is shown enclosed in a box.

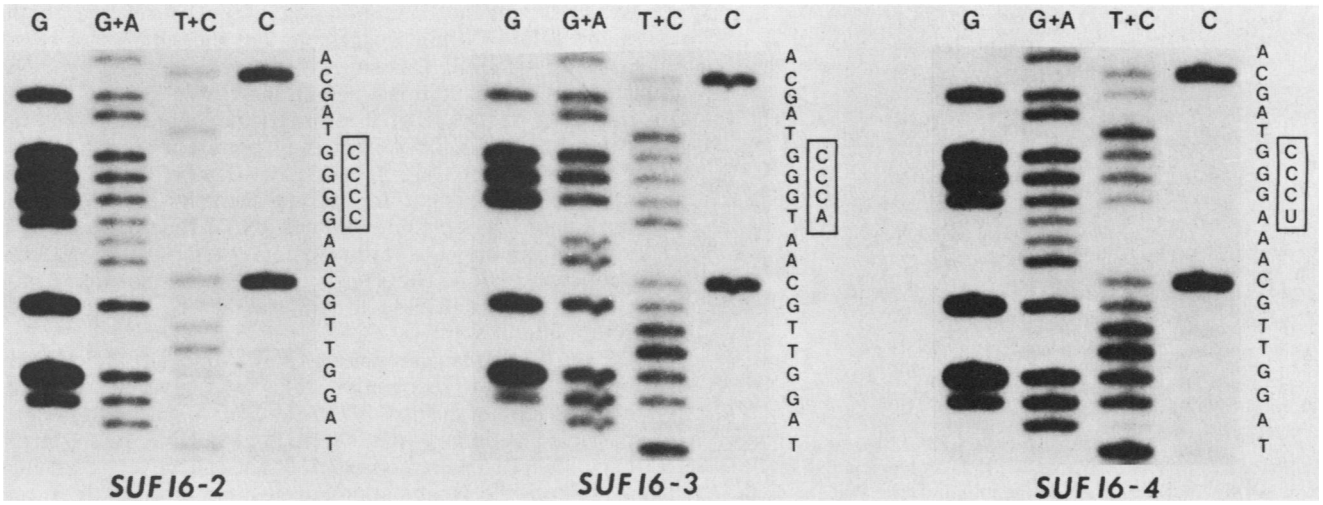

FIG. 2. DNA sequences of alternative *SUF16* alleles. The DNA sequences of the anticodon regions are shown for three suppressor alleles, *SUF16-2*, *SUF16-3*, and *SUF16-4*, derived from a strain carrying *his4-211* and *SUF16-1* (see the text). Comparison of these sequences with the corresponding anticodon regions of the wild-type and *SUF16-1* alleles determined by Gaber and Culbertson (14) indicates that the suppressor tRNAs contain base substitutions of G with C, A, or U at position 4 of the anticodon. The predicted 4-base anticodons are enclosed in boxes.

convention that the anticodon base is given first, followed by the codon base.

A nucleotide substitution in the *his4* message. His^+ revertants that contained a *his4-211*-linked mutation were tested to determine the extent of suppression by *SUF16-1*. Since these revertants were shown to contain an unaltered *SUF16-1-suf16*$^+$ duplication (see above), they were examined directly by monitoring growth on medium without histidine at 30 and 37°C. Each of three independent revertants exhibited a His^+ phenotype at both temperatures.

DNA fragments containing the *his4-211* region were cloned from each of the three revertants. The method of cloning and strategy for DNA sequence analysis were identical to those described for the original *his4-211* mutation (see above). Sequence analysis demonstrated that all three revertants contained an identical G:C-to-C:G transversion affecting position 4 of the *his4-211* 5′-GGGG-3′ codon (Fig. 4). These results establish the identity of a new allele, designated *his4-211R1*, that specifies the 4-base codon 5′-GGGC-3′. Furthermore, the results show that the *SUF16* tRNA acts during suppression at the site of the *his4-211R1* mutation and that a change in *his4-211* mRNA from 5′-GGGG-3′ to 5′-GGGC-3′ permits codon recognition by the 3′-CCCG-5′ anticodon of *SUF16-1*.

Position 4 base pairing relieves temperature-sensitive suppression. Using the available *SUF16* and *his4* alleles described above, we noted in preliminary in vivo suppression tests that certain combinations of 4-base anticodon-codon sequences resulted in temperature-sensitive suppression, whereas others were temperature independent (see above). Among three cases in which suppression was independent of temperature as indicated by growth on medium without histidine at 30 and 37°C, two, C/G and G/C, involved anticodon-codon position 4 base combinations with potential for base pairing. In contrast, the nonstandard base combination C/U also conferred temperature-independent suppression, whereas other nonstandard combinations conferred heat-sensitive suppression. The potential wobble pairs U/G and G/U conferred heat- and cold-sensitive suppression, respectively. Since 5′-GGGA-3′ codons at *his4* had not yet been identified, it was not possible to examine all 16 base combinations.

These observations prompted further studies in which we first asked whether the efficiency of suppression was higher in strains exhibiting temperature-independent suppression than in those exhibiting heat-sensitive suppression. This was accomplished by measuring *HIS4C*-encoded histidinol dehydrogenase activity in representative suppressor strains (see above). *HIS4C* activity reflects the extent to which polarity into the *his4C* region due to an upstream frameshift mutation in *HIS4A* or *HIS4B* is relieved by the action of a suppressor. When compared with *HIS4C* activity in a wild-type strain, the activity in a suppressor strain is a relative measure of the

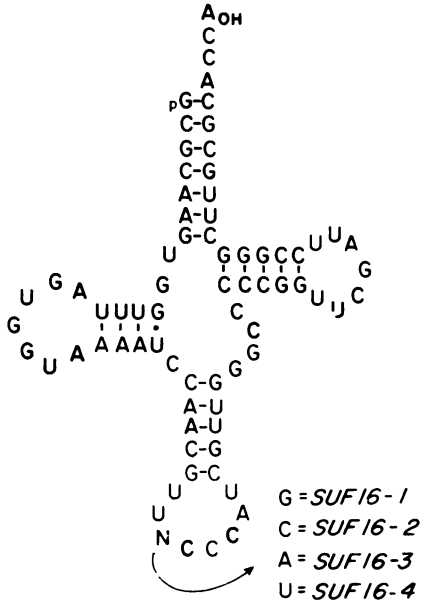

FIG. 3. Cloverleaf structure of *SUF16* suppressor tRNAs, showing the primary sequence of *SUF16* tRNA deduced from the DNA sequence (14). Base modifications that have been identified in the wild-type tRNA (40) have not been verified to be present in the suppressor tRNAs and are therefore not shown. The nth nucleotide represents position 4 of the anticodon for which all possible base substitutions have been identified.

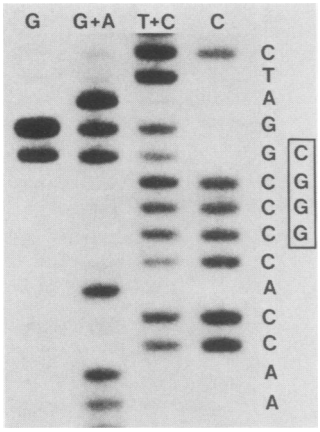

FIG. 4. DNA sequence of the *his4-211R1* mutation. The DNA sequence shown was compared with the correpsonding sequence of the *his4-211* allele (Fig. 1). The results indicate that the *his4-211R1* mutation derived from a strain carrying *his4-211* and *SUF16-1* (see the text) is a G:C-to-C:G transversion. The mutation results in a substitution of G with C in the mRNA at position 4 of the *his4-211* 4-base codon shown enclosed in a box.

percent transmission of ribosomes in the correct reading frame through the site of the frameshift mutation and is therefore proportional to the efficiency of suppression (5). These assays revealed that strains exhibiting temperature-independent suppression had efficiencies of suppression that ranged from 10 to 20%. Strains exhibiting heat-sensitive suppression had efficiencies that ranged from 1 to 10% (data not shown).

Since higher efficiencies of suppression appeared to result in temperature-independent suppression, we tested the hypothesis that position 4 bases having the potential for interaction might contribute enough stability to an anticodon-codon interaction to confer temperature-independent suppression. This was accomplished by analyzing His^+ revertants at 37°C in strain R564, which among other markers contains *SUF16-4* (3'-CCCU-5'), *his4-211* (5'-GGGG-3') and *leu2-3* (see above). The parental R564 strain exhibits heat-sensitive suppression of *his4-211*. According to the hypothesis being tested, a U/G wobble interaction at position 4 might be expected to result in temperature-independent suppression of *his4-211*. However, since U at position 3 in normal 3-base tRNA anticodons is usually modified in *S. cerevisiae* in a manner that prevents wobble (16), the phenotypic behavior of this strain may result from modification of position 4 U in the *SUF16-4* tRNA.

Temperature-independent His^+ revertants in strain R564, which occur at an approximate frequency of 10^{-8} per cell plated, were divided into two phenotypic classes based on the observed strength of suppression of the *leu2-3* frameshift mutation. Representative mutations of one class exhibited weak suppression of *leu2-3* in a manner identical to the parental R564 strain and were shown to map at the *his4* locus. A DNA fragment containing one of these mutations was cloned, and the nucleotide sequence was determined (see above). The mutation, designated *his4-211R2*, is located at position 4 of the *his4-211* codon and results in a change from 5'-GGGG-3' to 5'-GGGA-3' (Fig. 5). This change, which results in a U/A rather than a U/G position 4 base combination, confers temperature-independent suppression on medium lacking histidine.

Representative mutations of the other phenotypic class exhibited enhanced suppression of *leu2-3* relative to the parental R564 strain, suggesting that the suppressor tRNA itself was altered. This suggestion was reinforced by showing that these mutations map at the *SUF16* locus. Although the DNA sequence of these particular mutations was not determined, it is likely that they represent a change from *SUF16-4* (3'-CCCU-5') to *SUF16-2* (3'-CCCC-5'). This change would result in the replacement of the original position 4 U/G combination with a C/G base pair, which is known from previous results to confer temperature-independent suppression. These results suggest that position 4 base pairing may contribute to the stability of the anticodon-codon interaction.

Four-base codon recognition rules. A more complete summary of codon recognition rules was possible after the identification of the *his4-211R2* (5'-GGGA-3') codon since all possible position 4 variants were not available. Accordingly, haploid strains were constructed that collectively contained all 16 possible combinations of position 4 nucleotides, and the extent of suppression was examined in each strain by monitoring growth on medium lacking histidine (Table 1). These qualitative assays revealed that suppression was detectable in strains representing any position 4 base combinations except G/G. These results indicate that codon recognition does not require position 4 base pairing or wobble.

Qualitative comparison of the extent of suppression in each strain revealed a wide range in the efficiency of suppression. In most cases we attribute these differences to position 4 nucleotide combinations since all 4-base anticodons and codons except one (5'-GGGU-3' at the *his4-38* site) are located in the same tRNA or at the same site in the mRNA. It was possible to define five general categories that reflect different efficiencies of suppression: (i) lack of suppression, (ii) weak suppression at 30°C with no suppression at 37°C, (iii) strong suppression at 30°C with no suppression at 37°C, (iv) weak suppression at 30°C with strong suppression at 37°C, and (v) strong suppression at both temperatures.

Strains containing the position 4 nucleotide combinations G/A, U/G, and U/U exhibited heat-sensitive suppression with very weak growth on medium lacking histidine at 30°C. In all three cases, variants exhibiting a higher efficiency of suppression appeared at an approximate frequency of 10^{-4}

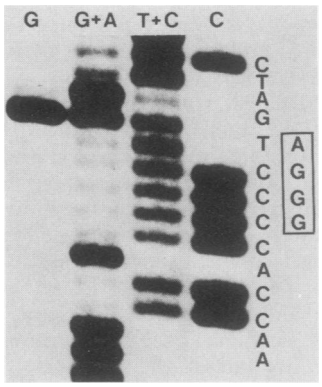

FIG. 5. DNA sequence of the *his4-211R2* mutation. The DNA sequence shown was compared with the corresponding sequence of the *his4-211* and *his4-211R1* alleles (Fig. 1 and 4). The results indicate that the *his4-211R2* mutation derived from a strain carrying *his4-211* and *SUF16-4* (see the text) is a G:C-to-A:T transition. The mutation results in a substitution of G with A in the mRNA at position 4 of the *his4-211* 4-base codon enclosed in a box.

TABLE 1. Codon recognition by frameshift suppressor tRNA[a]

Codon[b]	Anticodon[c]							
	Suf16-1 (CCC G)		SUF16-2 (CCC C)		SUF16-3 (CCC A)		SUF16-4 (CCC U)	
	30°C	37°C	30°C	37°C	30°C	37°C[d]	30°C	37°C
his4-211 (GGG G)	−(L)	−(L)	+	+	+	NT	±(H)	−(L)
his4-211R1 (GGG C)	+	+	+	−(L)	+	NT	+	−(H)
his4-211R2 (GGG A)	±(H)	−(L)	+	−(L)	+	NT	+	+
his4-38 (GGG U)	±(H)	+	+	+	+	NT	±(H)	−(H)

[a] Suppression was assayed by monitoring growth on medium lacking histidine. −, No detectable suppression; ±, weak suppression after 3 to 4 days; +, strong suppression after 1 to 2 days. (H) and (L) refer to high and low frequency reversion, which has been shown to result from chromosome III disomy and from position 4 base substitution, respectively (see the text).

[b] All 4-base codons listed are located at the site of the *his4-211* mutation (position +738 to 741 in *his4B*) except 5′-GGGU-3′, which is located at the site of the *his4-38* mutation (position +175 to 176 in *his4A*) (10,11; this study).

[c] Anticodon sequences were deduced from DNA sequences of cloned suppressor genes (see the text). The sequences as drawn disregard the possibility of base modification (see the text).

[d] NT, Suppression was not assayed at 37°C because *SUF16-3* confers temperature sensitive growth on YEPD medium (see the text).

per cell plated that were superimposed on the background of weak growth. Genetic analysis of these variants revealed that N+1 disomy for chromosome III was the genetic event responsible for the higher level of suppression (see above). Since the *his4* and *SUF16* genes are located on chromosome III (15, 25), these results suggest that the efficiency of suppression depends in part on the dosage of genes encoding the tRNA or mRNA or both. Since the phenotypically His^- strain R453 that contains the position 4 nucleotides G/G does not give rise to chromosome III disomes, the effect of a G/G nucleotide combination is qualitatively different from other base combinations that result in weak suppression enhanced by disomy.

Three strains containing the position 4 nucleotide combinations C/C, C/A, and U/C exhibited heat-sensitive suppression with strong growth on medium lacking histidine at 30°C. One strain containing a position 4 G/U nucleotide combination exhibited a cold-sensitive phenotype with more efficient suppression at 37 than at 30°C. His^+ revertants of this strain that arise at high frequency at 30°C were shown to be the result of chromosome III disomy. An additional four strains containing the combinations G/C, C/G, C/U, and U/A exhibited temperature-independent suppression. All of these combinations have base pairing potential with the exception of C/U.

It was not possible to examine suppression at 37°C in the four strains carrying *SUF16-3* (3′-CCCA-5′). Each of these strains exhibited temperature-sensitive growth on fully supplemented YEPD medium at 37°C, making it difficult to assess the extent of suppression of *his4* frameshift mutations. The following observations suggest that the temperature-sensitive phenotype is conferred by the *SUF16-3* mutation.

Integrative transformation of temperature-independent yeast strains carrying *leu2-3*, *suf16*$^+$, and *his4-38*, *his4-211*, *his4-211R1*, or *his4-211R2* with a plasmid carrying the *SUF16-3* gene yielded two classes of transformants: (i) 14 colonies that were phenotypically Leu^+ His^+ (*SUF16-3*) and temperature sensitive and (ii) 12 colonies that were Leu^- His^- (*suf16*$^+$) and temperature independent. The latter class of transformants represent gene coconversions of the two phenotypes. Representatives of the former class were analyzed further by first selecting for loss of the duplication generated by transformation (see above), resulting in haploid strains containing a single copy of the *SUF16-3* suppressor gene. When these strains were used, it was found that the temperature-sensitive phenotype is dominant in *suf*$^+$/*SUF16-3* heterozygous diploids. In addition, reversion of the temperature-sensitive phenotype in these haploid strains frequently caused altered suppression. Among 95 revertants examined, 72 showed complete loss of suppression, which could be due to gene conversion directed by unlinked members of the *SUF16* multi-tRNA gene family. Most of the remaining revertants exhibited various degrees of partial loss of suppression and are undoubtedly the result of mutations that affect the efficiency of suppression.

Coconversion, dominance, and coreversion all suggest that the temperature-sensitive phenotype is caused by the *SUF16-3* allele. Although we do not understand why *SUF16-3* confers temperature-sensitive growth, whereas other alleles of the same gene do not, it is interesting to note that *S. cerevisiae* probably does not contain the wild-type counterpart (3′-CCA-5′ anticodon) of the *SUF16-3* tRNA. It is possible that this unique feature is in some way responsible for disruption of normal translation.

Overall, our results support the generalization that nonstandard base combinations are associated with lower efficiencies of suppression, whereas base combinations with potential for position 4 base interactions are associated with higher efficiencies of suppression. Three demonstrable exceptions to this generalization are based on the observation of weak, temperature-sensitive suppression for the potential U/G wobble pair, cold-sensitive suppression for the potential G/U wobble pair, and efficient, temperature-independent suppression for the nonstandard C/U pair.

Codon position effect. The following experiments were performed to determine whether the position of a 4-base codon in the *his4* gene influences the efficiency of suppression. Position 4 variants at the site of the *his4-519* mutation in the *his4A* region were isolated to obtain a 4-base codon identical to one of the 4-base codons located at the site of the *his4-211* mutation in *his4B*. Such position 4 variants were isolated in a manner similar to that described for *his4-211* variants. His^+ revertants were analyzed in the phenotypically His^- strain R379, which among other markers carries *his4-519* (5′-GGGG-3′) and *SUF16-1* (3-′CCCG-5′) (see above). A DNA fragment containing the *his4-519* region from one temperature independent His^+ revertant of this

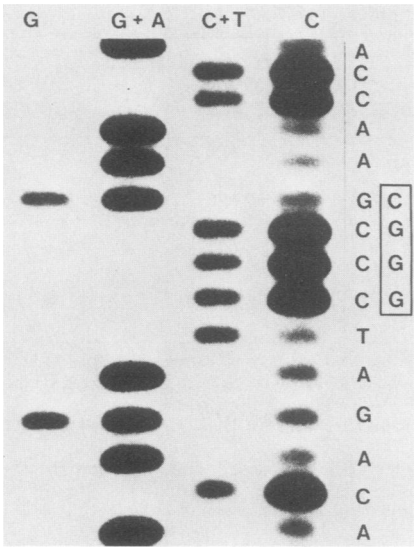

FIG. 6. DNA sequence of the *his4-519R1* mutation. The DNA sequence shown was compared with the corresponding wild-type sequence of the *HIS4A* region and the sequence of the *his4-519* mutation determined by Donahue et al. (10, 11). The results indicate that the *his4-519R1* mutation derived from a strain carrying *his4-519* and *SUF16-1* (see the text) is a G:C-to-C:G transversion. The mutation results in a substitution of G with C in the mRNA at position 4 of the *his4-519* 4-base codon shown enclosed in a box.

strain was cloned and examined by DNA sequence analysis (Fig. 6). This mutation, designated *his4-519R1*, results in a G:C-to-C:G transversion affecting position 4 of the *his4-519* 5′-GGGG-3′ codon. The resulting 5′-GGGC-3′ codon is identical in sequence to the *his4-211R1* allele, but the two codons are separated by 243 bases.

The influence of codon location was examined by comparing the relative efficiencies with which *his4-519R1* and *his4-211R1* are suppressed by alleles of the *SUF16* locus. Two tests were performed: (i) qualitative observation of growth on medium without histidine and (ii) quantitative measurement of *HIS4C*-encoded histidinol dehydrogenase activity resulting from suppression.

To provide a qualitative comparison, three of the four alleles of *SUF16* were examined for their ability to suppress each codon. As expected, *SUF16-1* (3′-CCCG-3′) suppressed both mutations efficiently, such that no distinction in the level of suppression was possible, presumably due to position 4 G/C base pairing. Tests with two other *SUF16* alleles that generate position 4 base combinations C/C and U/C revealed obvious growth differences reflecting different levels of suppression of each 4-base codon. By using the phenotypic designations of Table 1, suppression of *his4-519R1* by these *SUF16* alleles resulted in a His^+ phenotype, whereas suppression of *his4-211R1* resulted in a $His^{\pm}$ phenotype at 30°C. Our results suggest that *his4-519R1* is suppressed less efficiently than *his4-211R1* despite sequence identity of these 4-base codons.

To measure the difference in efficiency of suppression quantitatively, the extent to which suppression relieves polarity of the frameshift mutations into *HIS4C* was assayed in strains carrying *SUF16-2* and either *his4-519R1* or *his4-211R1* (see above for rationale, method of assay, and experimental controls.) In both cases position 4 is occupied by a C/C pair. *SUF16-2* suppresses *his4-211R1* more efficiently than *his4-519R1* (Fig. 7); by comparison with *HIS4C* activity in a wild-type strain, the mean efficiencies of suppression are 6 and 1%, respectively. These results suggest that the location of a suppressible codon within mRNA can influence the apparent efficiency of suppression.

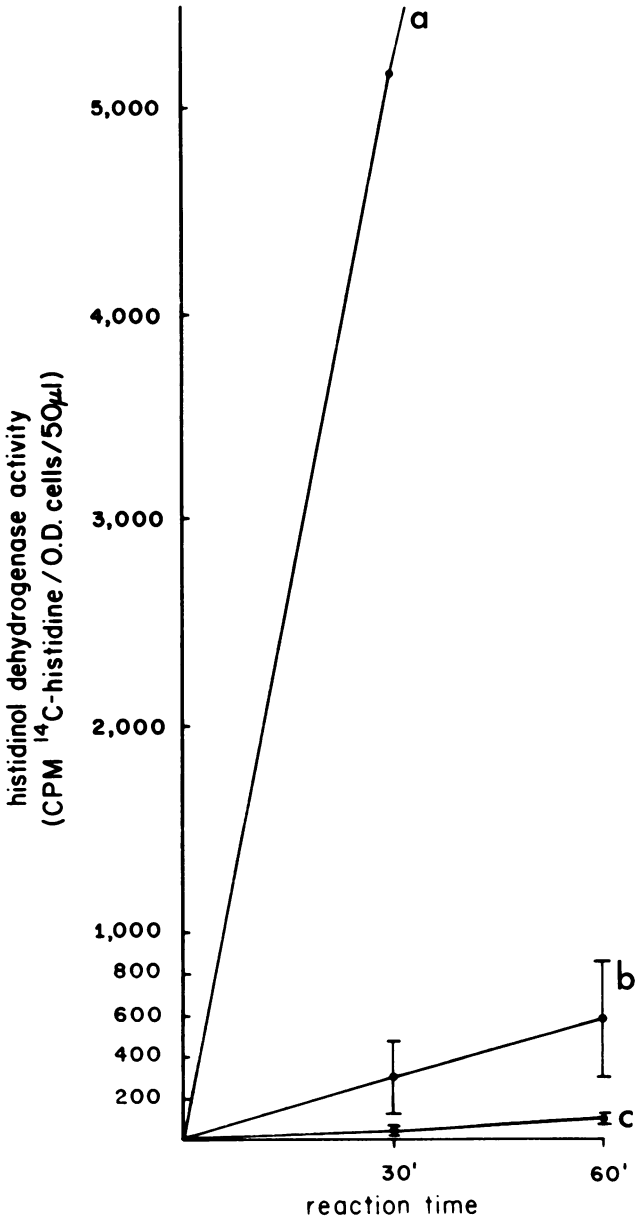

FIG. 7. Codon location influences efficiency of suppression. The efficiency of suppression by *SUF16-2* was determined in two sets of strains carrying the identical 4-base codons *his4-519R1* and *his4-211R1*, which are located in *his4A* (+493 to 495) and *his4B* (+738 to 741), respectively. Efficiency of suppression was determined by assaying the activity of the downstream *HIS4C*-encoded histidinol dehydrogenase (see the text). The assay measures the extent to which polarity of these frameshift mutations into the downstream *HIS4C* region is relieved by the action of the suppressor (see the text). The 1-h time point for the *suf16*$^+$ *HIS4*$^+$ strain is not shown in the figure, but the reaction is linear for more than 1 h. The average efficiencies of suppression relative to the wild-type strain are 6% for *his4-211R1* and 1% for *his4519R1*. The controls for this experiment and statistical methods for analyzing the data are described in the text. a, *suf16*$^+$ *HIS4*$^+$; b, *SUF16-2 his4-211R1*; c, *SUF16-2 his4-519R1*.

DISCUSSION

Altered tRNAs that suppress +1 frameshift mutations in *Salmonella typhimurium* have been shown to contain an extra nucleotide within an anticodon consisting of either like or unlike bases (3, 30, 33). Suppressor tRNAs of the former type, such as *sufD* (anticodon 3'-CCCC-5') (31), may cause a shift to the +1 reading frame through formation of 4 base pairs with mRNA. Alternatively, such tRNAs may form only 3 base pairs involving either the first or last 3 of 4 identical codon bases potentially available for interaction. These theoretical considerations suggest that 4-base codon recognition by suppressors such as *sufD* may resemble conventional 3-base decoding regarding a requirement for base pairing or wobble at the 5'-most anticodon position.

Suppressor tRNAs having unlike anticodon bases, such as the threonine tRNA encoded by *sufJ* (3'-UGGN-5'), probably do not utilize the ambiguous 3-base reading mechanism described above since *sufJ*-suppressible codons 5'-ACCN-3' cannot be read in two alternate frames of reference (3). A suppressor of this type might require base interactions only at the first three positions, whereas the noninteracting fourth anticodon base could force translation into the +1 phase. *sufJ* might therefore disobey conventional 3-base decoding rules and allow for nonstandard position 4 base combinations.

Since the *sufD* and *sufJ* genes encode structurally dissimilar tRNAs, it has not been possible to separate the influence of anticodon bases from the influence of other features of tRNA structure or expression that might affect suppression. To evaluate the mechanism of frameshift suppression in a manner that attempts to circumvent this problem, we have isolated four alternative suppressor alleles of a single yeast glycine tRNA gene, *SUF16*, each of which specifies one of four possible 3'-CCCN-5' anticodons. The primary sequences of the tRNAs encoded by these alleles are otherwise identical. By assessing the phenotypic behavior of these alleles in strains containing each of four possible 5'-GGGN-3' glycine codons at the *his4* locus, it has been possible to define in more exact terms those features of the tRNA-mRNA interaction that determine codon recognition.

SUF16 tRNA shares some structural similarities with *sufD*. In particular, both are glycine tRNAs that contain cytidine residues in the first three anticodon positions. Nonetheless, our results show that the phenotypic behavior of *SUF16* suppressor alleles (Table 1) resembles the behavior of *sufJ* rather than that of *sufD*. *SUF16*-mediated suppression was detected in 15 of the 16 anticodon-codon position 4 base combinations tested, including many nonstandard combinations which lack potential for base pairing or wobble. We failed to detect suppression in only one case in a strain containing the position 4 combination G/G. Lack of suppression could be due to an efficiency of suppression below limits of detection. This idea has been addressed by asking whether suppression could be detected under conditions of increased dosage of the appropriate tRNA gene. Unlike some position 4 base combinations in which a low efficiency of suppression can be enhanced by increased gene dosage of tRNA or mRNA, no suppression was observed in a strain containing five plasmid copies of the *SUF16-1* allele (3'-CCCG-5') and one copy of *his4-211* (5'-GGGG-3') (B. Hyman, personal communication). Thus, it is likely that the G/G combination confers an efficiency of suppression far below that of other combinations. The phenotype of strains containing G/G may result from active interference of the tRNA-mRNA interaction by this position 4 base combination rather than from the inability of G/G to form hydrogen bonds.

In general, our results support the conclusion that position 4 base pairing or wobble are not required for a successful interaction leading to suppression. Even the *SUF16-2* allele, which contains an anticodon identical to that of *sufD* consisting of like bases (3'-CCCC-5'), disobeys pairing and wobble rules at position 4. These results suggest that it may be necessary to consider factors other than the anticodon-codon interaction in evaluating the specificity of *sufD*.

Our results also indicate that position 4 nucleotides can influence the efficiency of suppression. Efficient, temperature-independent suppression results whenever position 4 bases have the potential for interaction, whereas nonstandard base combinations result in less efficient, heat-sensitive suppression. There are three exceptions to these generalizations. The U/G combination, which in theory is capable of wobble, confers heat-sensitive suppression. The G/U combination, which in theory is also capable of wobble, confers cold-sensitive suppression. In addition, the nonstandard C/U combination confers temperature-independent suppression. The first exception might be explained by the possible presence of a uridine modification at position 4 that prevents wobble (see reference 16). This hypothesis has not yet been tested. The reasons for the exceptional phenotypes of the G/U and C/U combinations are not understood but might be explained by codon position effects (see below).

Based on these data we propose a model in which 4-base translocation of mRNA on the ribosome is directed primarily by the number of nucleotides in the anticodon loop. This mechanism would most likely be executed through recognition of some aspect of tRNA conformation (see reference 39). In addition to this underlying mechanism, our data suggest that the efficiency of suppression is strongly influenced by the potential for interaction of position 4 nucleotides.

We wish to point out two qualifications to these interpretations. First, all of our interpretations are based on primary tRNA sequences deduced from DNA sequences. Since the *SUF16* gene contains no intervening sequence, we are confident that the primary *SUF16* tRNA sequence (Fig. 3) is correct. However, we have not considered the effect of base modifications, particularly those which may reside in the anticodon of *SUF16* tRNAs encoded by different alleles. For example, inspection of Table 1 reveals two cases in which reciprocal base combinations result in different phenotypes: G/U versus U/G and C/U versus U/C. One possible explanation for these differences is that the position 4 base is modified in the tRNA but not in the reciprocal case in which the same base resides in the message.

The second qualification stems from the observation that the codon position within the *his4* message can influence the efficiency of suppression. Three of the four codons used in suppression tests reside at the position of *his4-211* in *his4B*, but the fourth (5'-GGGU-3') resides at *his4-38* in *his4A*. We have compared suppression of *his4-38* with sequenced frameshift mutations that generate 5'-GGGU-3' codons at other positions in the *his4* and *leu2* genes (unpublished data, this laboratory). These tests indicate that the pattern of suppression of *his4-38* by *SUF16* alleles resembles the pattern of suppression observed for some but not all of the 5'-GGGU-3' codons examined. Therefore, we cannot unambiguously eliminate the possibility that some of the exceptional cases might be explained if suppression of *his4-38* is inhibited or enhanced relative to *his4-211* alleles by virtue of its position.

The underlying cause of the codon position effect observed by comparing suppression of *his4-519R1* and *his4-211R1* is unknown. The position effect could result from

differential interference in the assembly of the multifunctional *his4* dimeric protein (20) by truncated polypeptides translated out of phase. This "poison subunit" explanation for position effect is unlikely on the grounds that the *his4-519R1* and *his4-211R1* truncated polypeptides terminate at UAG stop codons at positions +567 in *his4A* and +795 in *his4B*, respectively. Interference in dimer formation is unlikely since the major contact points for dimer formation are located in *his4C*. The latter conclusion stems from the fact that deletion of the entire *his4A* and *his4B* region has no effect on the activity of a dimeric *his4C* region (12, 20).

Another explanation is that mRNAs containing +1 insertions at different positions are differentially stable, as has been shown for mRNAs containing amber nonsense mutations at different positions in the *ura3* gene (22). Nonsense mutations at *ura3* showed a polar effect in which those mutations closest to the 3′ end of the gene conferred the greatest degree of mRNA stability. Although our results showing weaker suppression of *his4-519RI* versus *his4-211RI* are consistent with the interpretation of such a polar effect of mRNA stability, it will be necessary to examine additional +1 insertions in *his4* to verify the existence of polarized efficiency of suppression.

Finally, it is possible that codon position effect is due to the influence of surrounding sequences that provide either good or bad "context" during translation. The influence of codon context on the efficiency of nonsense suppression has been demonstrated in *S. typhimurium* (2), in which it was found that enhanced suppression could result from base substitution in the first codon downstream from the nonsense codon. Since these mutations resulted in an amino acid substitution, it is possible that context may stem from tRNA-tRNA interactions on the ribosome. Further investigation will be necessary to determine whether similar codon context effects influence frameshift suppression.

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LITERATURE CITED

1. **Atkins, J. F., R. J. Gesteland, B. R. Reid, and C. W. Anderson.** 1979. Normal tRNAs promote ribosomal frameshifting. Cell **18**:1119–1131.
2. **Bossi, L., and J. R. Roth.** 1980. The influence of codon context on genetic code translation. Nature (London) **286**:123–127.
3. **Bossi, L., and J. R. Roth.** 1981. Four-base codons ACCA, ACCU, and ACCC are recognized by frameshift suppressor *sufJ*. Cell **25**:489–496.
4. **Ciesla, Z., F. Salvatore, J. R. Broach, S. Artz, and B. N. Ames.** 1975. A sensitive radiochemical assay for histidinol dehydrogenase. Anal. Biochem. **63**:44–55.
5. **Culbertson, M. R., L. Charnas, M. T. Johnson, and G. R. Fink.** 1977. Frameshifts and frameshift suppressors in *Saccharomyces cerevisiae*. Genetics **86**:745–764.
6. **Culbertson, M. R., and S. A. Henry.** 1973. Genetic analysis of hybrid strains trisomic for the chromosome containing a fatty acid synthetase gene complex (*fas1*) in yeast. Genetics **75**:441–458.
7. **Culbertson, M. R., K. M. Underbrink, and G. R. Fink.** 1980. Frame shift suppression in *Saccharomyces cerevisiae*. II. Genetic properties of group II suppressors. Genetics **95**:833–853.
8. **Cummins, C. M., T. F. Donahue, and M. R. Culbertson.** 1982. Nucleotide sequence of the *SUF2* frameshift suppressor gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **79**:3565–3569.
9. **Cummins, C. M., R. F. Gaber, M. R. Culbertson, R. Mann, and G. R. Fink.** 1980. Frameshift suppression in *Saccharomyces cerevisiae*. III. Isolation and genetic properties of group III suppressors. Genetics **95**:855–875.
10. **Donahue, T. F., P. J. Farabaugh, and G. R. Fink.** 1981. Suppressible glycine and proline four base codons. Science **212**:455–457.
11. **Donahue, T. F., P. J. Farabaugh, and G. R. Fink.** 1982. The nucleotide sequence of the *HIS4* region of yeast. Gene **18**:47–59.
12. **Fink, G. R., and C. Styles.** 1974. Gene conversion of deletions in the *HIS4* region of yeast. Genetics **77**:231–244.
13. **Fox, T. D., and B. Weiss-Brummer.** 1980. Leaky +1 and −1 frame shift mutations at the same site in a yeast mitochondrial gene. Nature (London) **288**:60–63.
14. **Gaber, R. F., and M. R. Culbertson.** 1982. The yeast frameshift suppressor gene *SUF16-1* encodes an altered tRNA containing the four-base anticodon 3′-CCCG-5′. Gene **19**:163–172.
15. **Gaber, R. F., and M. R. Culbertson.** 1982. Frameshift suppression in *Saccharomyces cerevisiae*. IV. New suppressors among spontaneous co-revertants of the group II *his4-206* and *leu2-3* frameshift mutations. Genetics **101**:345–367.
16. **Guthrie, C., and J. Abelson.** 1982. Organization and expression of tRNA genes in *Saccharomyces cerevisiae*, p. 487–528. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: Metabolism and gene expression, vol. 2. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
17. **Hicks, J. B., and G. R. Fink.** 1977. Identification of chromosomal location of yeast DNA from hybrid plasmid pYeleu10. Nature (London) **269**:265–267.
18. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. (U.S.A.) **75**:1229–1233.
19. **Kastelein, R. A., E. Ramaut, W. Fiers, and J. Van Duin.** 1982. Lysis gene expresison of RNA phage MS2 depends on a frameshift during translation of the overlapping coat protein gene. Nature (London) **295**:35–41.
20. **Keesey, J. K., R. Begelis, and G. R. Fink.** 1979. The product of the *his4* gene cluster in *Saccharomyces cerevisiae*, a trifunctional polypeptide. J. Biol. Chem. **254**:7427–7433.
21. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
22. **Losson, R., and R. LaCroute.** 1979. Interference of nonsense mutations with eucaryotic messenger RNA stability. Proc. Natl. Acad. Sci. U.S.A. **76**:5134–5137.
23. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labelled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
24. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
25. **Mortimer, R. K., and D. Schild.** 1981. Genetic map of *Saccharomyces cerevisiae*, p. 11–27, 641. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: Life cycle and inheritance, vol. 1. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
26. **Olson, M. V., K. Loughney, and B. D. Hall.** 1979. Identification of the yeast DNA sequences that correspond to specific tyrosine-inserting nonsense suppressor loci. J. Mol. Biol. **132**:387–410.
27. **Petes, T. D., J. R. Broach, P. C. Wensink, L. M. Hereford, G. R. Fink, and D. Botstein.** 1978. Isolation and analysis of recombinant DNA molecules containing yeast DNA. Gene **4**:37–49.
28. **Riddle, D., and J. R. Roth.** 1970. Suppressors of frameshift mutations in *Salmonella typhimurium*. J. Mol. Biol. **54**:131–144.
29. **Riddle, D., and J. R. Roth.** Frameshift suppressors. II. Genetic mapping and dominance studies. J. Mol. Biol. **66**:483–493.
30. **Riddle, D., and J. R. Roth.** 1972. Frameshift suppressors. III. Effect of suppressor mutations on tRNA. J. Mol. Biol. **66**:495–506.

31. **Riddle, D., and J. Carbon.** 1973. A nucleotide addition to be the anticodon of a glucine tRNA. Nat. New Biol. **242**:230–237.
32. **Roeder, G. S., and G. R. Fink.** 1980. DNA rearrangements associated with transposable element in yeast. Cell. **21**:239–249.
33. **Roth, J. R.** 1981. Frameshift suppression. Cell **24**:601–602.
34. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **76**:4951–4955.
35. **Shafer, B., I. Brearley, R. Littlewood, and G. R. Fink.** 1971. A stable aneuploid of *Saccharomyces cerevisiae*. Genetics **67**:483–495.
36. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1971. Methods in yeast genetics. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
37. **Smith, D. R., and J. M. Calvo.** 1980. Nucleotide sequence of the *E. coli* gene coding for dihydrofolate reductase. Nucleic Acids Res. **8**:2255–2274.
38. **Stinchcomb, A. T., M. Thomas, J. Kelly, E. Selker, and R. Davis.** 1980. Eukaryotic DNA segments capable of autonomous replication in yeast. Proc. Natl. Acad. Sci. U.S.A. **77**:4559–4563.
39. **Woese, C.** 1970. Molecular mechanism of translation: a reciprocating ratchet mechanism. Nature (London) **226**:817–820.
40. **Yoshida, M.** 1972. The nucleotide sequence of $tRNA^{gly1}$ from yeast. Biochem. Biophys. Res. Commun. **50**:779–784.