FRAMESHIFT SUPPRESSION IN *SACCHAROMYCES CEREVISIAE*. V. ISOLATION AND GENETIC PROPERTIES OF NONGROUP-SPECIFIC SUPPRESSORS

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ABSTRACT

Two classes of frameshift suppressors distributed at 22 different loci were identified in previous studies in the yeast *Saccharomyces cerevisiae*. These suppressors exhibited allele-specific suppression of +1 G:C insertion mutations in either glycine or proline codons, designated as group II and group III frameshift mutations, respectively. Genes corresponding to representative suppressors of each group have been shown to encode altered glycine or proline tRNAs containing four base anticodons.——This communication reports the existence of a third class of frameshift suppressor that exhibits a wider range in specificity of suppression. The suppressors map at three loci, *suf12*, *suf13*, and *suf14*, which are located on chromosomes *IV*, *XV*, and *XIV*, respectively. The phenotypes of these suppressors suggest that suppression may be mediated by genes other than those encoding the primary structure of glycine or proline tRNAs.

FRAMESHIFT mutations result from the addition or deletion of base pairs in a gene encoding a protein product. Mutations of this type usually render the gene product nonfunctional due to the inability of the translational apparatus to recognize the shift in reading frame. This results in an incorrect specification of amino acids and in termination of translation at the first nonsense codon encountered.

The correct reading frame can be restored in strains carrying frameshift mutations by several different compensatory mechanisms. In some instances suppressor mutations in genes external to that which carries the frameshift mutation have been shown to affect the structures of tRNAs, tRNA base modification enzymes or ribosomal proteins (RIDDLE and CARBON 1973; CUMMINS, DONAHUE and CULBERTSON 1982; GABER and CULBERTSON 1982a; RIDDLE and ROTH 1972; ATKINS 1980; GORINI 1974). To examine these mechanisms in eukaryotes, mutationally induced nontriplet reading systems have been developed in this laboratory by the isolation of external suppressors of frameshift mutations at the *his4* locus in *Saccharomyces cerevisiae*.

Two groups of externally suppressible frameshift mutations, designated group II and group III, have been identified among 2-methoxy-9-[-3-(ethyl-2-chloro-

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ethyl)aminopropylamino]acridine·2HCl (ICR-170)-induced *his4* alleles (CULBERTSON *et al.* 1977; CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b). Two representatives of the group II mutations, *his4-38* and *his4-519*, contain base insertions resulting in 5′-GGGU-3′ and 5′-GGGG-3′ mRNA sequences, respectively, in place of wild-type 5′-GGU-3′ and 5′-GGG-3′ glycine codons. Two representatives of the group III mutations, *his4-712* and *his4-713*, both contain 5′-CCCU-3′ mRNA sequences in place of wild-type 5′-CCU-3′ proline codons (DONAHUE, FARABAUGH and FINK 1981).

A third group of ICR-induced *his4* mutations, designated group I, is not suppressible by external suppressors of group II or group III frameshift mutations (CULBERTSON *et al.* 1977; CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b). Although this group may be heterogeneous in composition, sequencing data indicate that one of these mutations, *his4-506*, is the result of a +1 insertion in a wild-type 5′-CUG-3′ leucine codon, generating a four-base 5′-CCUG-3′ mRNA sequence (DONAHUE, FARABAUGH and FINK 1981). Thus, the group I mutations may represent insertions in codons other than glycine or proline.

In previous studies in this laboratory, extragenic suppressors distributed at 22 loci have been identified among revertants of group II and group III *his4* frameshift mutations (CULBERTSON *et al.* 1977; CULBERTSON, UNDERBRINK and FINK 1980; CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b). These suppressors have been divided into two classes that are distinguished phenotypically by their ability to exhibit group II-specific or group III-specific patterns of suppression. Molecular studies have shown that representative suppressors of these two classes encode altered glycine and proline tRNAs containing base insertions in the anticodon loops (CUMMINS, DONAHUE and CULBERTSON 1982; GABER and CULBERTSON 1982a). Thus, in the cases of group II-specific and group III-specific suppression, these representative examples indicate that suppression is mediated through mRNA:tRNA interactions.

This communication defines a new class of frameshift suppressors consisting of three different suppressor loci, *suf12*, *suf13*, and *suf14*. All three suppressor genes have been located on the yeast genetic map. These suppressors exhibit a nongroup-specific spectrum of suppression. *suf12* is able to cross-suppress certain frameshift and nonsense mutations. *suf13* and *suf14* cross-suppress representative frameshift mutations belonging to groups I, II and III. The phenotypes of these suppressors suggest that suppression may be mediated by genes other than those encoding the primary structures of glycine or proline tRNAs. Several possible molecular mechanisms of suppression consistent with the phenotypes of nongroup-specific suppressors are discussed.

MATERIALS AND METHODS

Yeast strains, genetic methods, and media: Some of the strains used in this study are listed in Table 1. Genetic markers, if not from this laboratory, were obtained from the Berkeley Stock Center. Genetic methods and nomenclature are those described in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and LAWRENCE 1971).

The following standard media were used: YEPD, which contains 2% Bactopeptone, 1% yeast extract, 2% glucose and 2% agar; MIN (minimal), which contains 6.7 g/liter Difco yeast nitrogen base without amino acids, 2% glucose and 2% agar; KAC (sporulation medium), which contains 1%

potassium acetate, 0.1% glucose, 1.25 g/liter yeast extract and 2% agar; YEPDG, which contains 2% Bactopeptone, 1% yeast extract, 2% glycerol (v/v), 0.1% glucose, 1.25 g/liter yeast extract and 2% agar. Synthetic complete medium contains the components of minimal medium plus adenine, uracil, tyrosine, phenylalanine, histidine, leucine, tryptophan, methionine, lysine, arginine and threonine. Purines, pyrimidines and amino acids were added at concentrations given in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and LAWRENCE 1971).

The segregation of auxotrophic markers in crosses was scored on synthetic complete medium lacking the appropriate purine, pyrimidine or amino acid. Petite (*pet*) mutants were scored on YEPDG medium. The *tra3* mutation (triazolealanine resistance) confers temperature sensitivity on YEPD at 37° and was scored on this basis (WOLFNER *et al.* 1975). The *prt1* mutation, which confers temperature sensitivity for protein synthesis, was scored at the restrictive temperature of 37° on YEPD.

ICR-170 mutagenesis: ICR-170 was obtained from Polysciences, Inc. The *his4-713*, *met2-1* and *leu2-3* mutations used in this study were induced with ICR-170 according to the method of BRUSICK (1970) as modified by CULBERTSON *et al.* (1977). ICR-induced coreversion of *his4-713* and *met2-1* was performed as described by CULBERTSON, UNDERBRINK and FINK (1980).

Isolation of suppressors: Revertants harboring suppressors of independent origin were isolated in strains 852 and 853 (Table 1) as follows. Single colonies were isolated on YEPD plates. Cells from individual colonies were suspended in culture tubes containing 3 ml of YEPD and grown overnight with shaking at 30° to a density of 2×10^8 cells/ml. Aliquots (0.4 ml) of each culture were spread on four different types of selective media lacking either histidine, methionine, leucine or tryptophan. Colonies from these plates that exhibited coreversion for at least two markers were chosen for subsequent analysis. To ensure that isolates were of independent origin, colonies from the same culture tube were chosen for analysis only if they differed in phenotype.

Genetic mapping: Mapping was accomplished by crossing strains carrying the suppressors with a series of ten mapping strains carrying signal markers distributed throughout most of the yeast genome (GABER and CULBERTSON 1982b). The two mapping strains that were shown to carry markers linked to the suppressors isolated in this study are listed in Table 1. It was possible to determine the segregation of the suppressors since all of these crosses were homozygous for the suppressible *leu2-3* mutation.

Standard linkage values were derived from tetrad data using the equation X (in centimorgans, cM) = 50 [tetratype asci + 6 (nonparental ditype asci)]/total asci (PERKINS 1949). Gene order in multipoint crosses was determined by analyzing recombinant asci containing crossovers in the regions of interest. In some crosses the suppressors were tested for linkage to a centromere using the equation (PERKINS 1949):

$$\frac{\text{tetratype asci}}{\text{total asci}} = X + Y - 3/2\,(XY)$$

where X is the second division segregation frequency of the centromere-linked marker *trp1* (0.45) (HAWTHORNE and MORTIMER 1960), and Y is the second division segregation frequency of the suppressor.

Spectrum of suppression: Strains used in suppression tests are listed in Table 1. In all cases the ability of a suppressor to suppress a particular marker was tested by tetrad analysis. Each cross was heterozygous for a suppressor and a potentially suppressible mutation. When a mutation failed to segregate 2:2 in these crosses, as indicated by an excess of prototrophic spore colonies, it was regarded as suppressible. Suppression was then confirmed in crosses homozygous for the mutation in question and heterozygous for the suppressor, in which case a 2:2 segregation was expected.

RESULTS

Isolation and distribution of the suppressors

Coreversion of his4-713 *and* met2-1: The original intention of this work was to identify new suppressors of group III frameshift mutations (+1 G:C insertions in proline codons). A previous study had shown that the most convenient

TABLE 1

List of strains

Strain[a]	Genotype	Source
Parental strains from which suppressors were derived:		
836	*his4-713*(III) *met2-1*(II+III) *lys2 MAT1α*	M. CULBERTSON
852	*his4-713*(III) *met2-1*(II+III) *leu2-3*(II) *lys2 trp1-1*(UAG) *MAT1***a**	M. CULBERTSON
853	*his4-713*(III) *met2-1*(II+III) *leu2-3*(II) *lys2 trp1-1*(UAG) *MAT1α*	M. CULBERTSON
Mapping strains:		
A236-57B	*leu2-3*(II) *trp1 met4 aro7 lys11 his3 SUC2 MAL3 can1 MAT1***a**	L. MATHISON
A230-2C	*leu2-3*(II) *pet2 arg4 ade8 aro1C trp4 rna3 MAT1***a**	L. MATHISON
Strains carrying frameshift and nonsense mutations:		
76	*his4-506*(I) *MAT1α*	M. CULBERTSON
527	*his4-520*(I) *MAT1α*	M. CULBERTSON
503	*his4-208*(I) *MAT1α*	M. CULBERTSON
493	*his4-707*(I) *MAT1α*	M. CULBERTSON
533	*his4-717*(I) *MAT1α*	M. CULBERTSON
513	*his4-507*(I) *MAT1***a**	M. CULBERTSON
524	*his4-518*(I) *MAT1***a**	M. CULBERTSON
R43	*his4-206*(II) *leu2-3*(II) *MAT1α*	M. CULBERTSON
78	*his4-38*(II) *MAT1α*	M. CULBERTSON
905	*his4-519*(II) *arg4-20*(II) *leu2-3*(II) *leu2-112*(I) *MAT1α*	M. CULBERTSON
82	*his4-504*(II) *MAT1α*	M. CULBERTSON
507	*his4-211*(II) *ade2-1*(UAA) *MAT1α*	M. CULBERTSON
120	*his4-712*(III) *ade2-1*(UAA) *MAT1α*	M. CULBERTSON
DC1	*leu2-2*(UGA) *arg4-17*(UAA) *lys1-1*(UAA) *met8-1*(UAG) *trp1-1*(UAG) *MAT1***a**	D. CHALEFF
145	*his4-644*(UAA) *MAT1α*	G. FINK
95	*his4-580*(UAG) *HOL1 MAT1α*	G. FINK
38	*his4-260*(UGA) *MAT1α*	G. FINK

[a] ICR-induced frameshift mutations are designated by a roman numeral in parentheses to indicate the group to which each mutation has been assigned (see CULBERTSON *et al.* 1977; GABER and CULBERTSON 1982). Nonsense mutations are designated as UAA, UAG or UGA, corresponding to ochre, amber and opal mutations, respectively.

approach to the isolation of suppressors involves coreversion of frameshift mutations in two genes conferring different auxotrophic requirements (CULBERTSON *et al.* 1977). Corevertants that arise as the result of external suppression of both markers can thus be distinguished from internal revertants in one or the other of the two mutant genes conferring the auxotrophic requirements.

Although well characterized group III frameshift mutations were available at the *his4* locus, no group III mutations in genes other than *his4* had been isolated prior to this study. Since at least one such mutation was required in order to develop an appropriate coreversion system, the wild-type strain S288C was mutagenized with ICR-170, and auxotrophic mutants were recovered and tested for their reversion and suppression properties. One mutant, which was shown by complementation and genetic mapping to be an allele of the *met2* locus

(*met2-1*), was found to corevert with the group III *his4-713* (5′-CCCU-3′) mutation after mutagenesis of a haploid double mutant strain with ICR-170. Among 22 ICR-induced corevertants that were analyzed genetically, all were found to contain alleles of the group III-specific, proline tRNA-encoding *SUF2* gene (CUMMINS *et al.* 1980; CUMMINS, DONAHUE and CULBERTSON 1982). Thus, *met2-1* was tentatively classified as a group III frameshift mutation.

Subsequently, *met2-1* was examined for its ability to be suppressed by other group III-specific suppressors (Table 2). Among the group III-specific suppressors, *met2-1* was suppressed by *SUF10* as well as *SUF2-1*. However, other group III-specific suppressors, including *SUF7*, *SUF8*, *SUF9* and *suf11*, failed to suppress *met2-1*. These latter suppressors have also been shown not to suppress *his4-712*, a group III frameshift mutation that is known to contain the same codon change as the suppressible *his4-713* mutation (5′-CCU-3′ → 5′-CCCU-3′) (CUMMINS *et al.* 1980; DONAHUE, FARABAUGH and FINK 1981). In this respect *met2-1* and *his4-712* behave similarly in that they are suppressed by some but not all of the group III-specific suppressors.

Surprisingly, *met2-1* was also suppressed by all of the group II-specific suppressors (Table 2). Thus, by virtue of its ability to be suppressed by representative suppressors of both groups, *met2-1* cannot be classified according to previously defined criteria. This is the first example in yeast of a frameshift mutation with this phenotype. A possible molecular mechanism for cross-suppression of *met2-1* is discussed in a subsequent section.

Analysis of revertants: In a preliminary study, a recessive suppressor, designated *suf12*, was obtained by coreversion of *his4-713* and *met2-1*. Unlike previously described group III-specific suppressors, which fail to suppress nonsense mutations, this suppressor was shown to cross-suppress the UAG mutation *trp1-1*. The *suf12* mutation was shown to be linked to the *aro1* locus on chromosome *IV*, whereas none of the previously identified frameshift suppressors map in this region. The distinguishing features of *suf12* prompted a more systematic investigation of the phenomenon of cross-suppression. Additional suppressors were analyzed in two separate studies.

In the first of these investigations no alleles of *suf12* were identified, but suppressor mutations mapping at two other new loci were recovered. Thirty-five spontaneous His^+ Met^+ corevertants of strain 836 (Table 1) were isolated. These corevertants were not necessarily of independent origin since some were derived from the same clonal isolates. Each of the corevertants was crossed with a strain carrying *his4-713 MET2*$^+$ *leu2-3*, and the purified diploids were tested for dominance. All of the diploids were His^- in phenotype, suggesting that if the corevertants carried external suppressors they were phenotypically recessive in a *his4-713* homozygous background.

One of these diploids was sporulated, and tetrads were analyzed. The observed segregation patterns were as follows: His^+:His^-, 2:2; Met^+:Met^-, 2:2, 4:0 and 3:1; and Leu^+:Leu^-, 2:2, 4:0, and 3:1. These results could be interpreted to mean that the corevertant carried an external suppressor capable of cross-suppressing the group III *his4-713* mutation, *met2-1*, and the group II *leu2-3* mutation. To test this hypothesis, a diploid was constructed that was homozy-

TABLE 2

Cross-suppression of met2-1 *by group II- and group III-specific suppressors*

Type of suppressor[b]	Suppression of:[a]				
	his4-519 5′-GGGG-3′	*his4-38* 5′-GGGU-3′	*his4-713* 5′-CCCU-3′	*his4-712* 5′-CCCU-3′	*met2-1* unknown
Group II-specific					
Subclass 1 (1, 3, 4, 5, 6, 17)	+	+	−	−	+
Subclass 2 (15, 16, 18, 19, 20, 21, 22, 23, 24, 25)	−	+	−	−	+
Group III-specific					
Subclass 1 (2, 10)	−	−	+	+	+
Subclass 2 (7, 8, 9, 11)	−	−	+	−	−

[a] The suppresible four base codons for each frameshift mutation are shown, if known. + indicates suppression; − indicates no detectable suppression after ten days of incubation.

[b] Numbers in parentheses indicate gene numbers for suppressors belonging to each subclass.

gous for *his4-713*, *met2-1* and *leu2-3* and heterozygous for the putative external suppressor. In tetrads derived from this diploid, the His, Met and Leu phenotypes cosegregated 2 His^+Met^+ Leu^+:2 His^- Met^- Leu^- spores per tetrad. This demonstrated that a single suppressor mutation, designated *suf13*, was cross-suppressing both group II and group III mutations.

The remaining 34 corevertants were examined by complementation and segregation analyses. First, a strain carrying *his4-713* and *suf13-1* was crossed with each of the remaining corevertants. The ability of each recessive suppressor mutation to complement *suf13-1* was tested in these diploids. In this type of complementation test, a His^+ phenotype in the heterozygous diploid suggests a negative complementation response, whereas a His^- phenotype indicates a positive complementation response.

A negative complementation response was observed in 29 of the diploids, as indicated by a His^+ phenotype. When tetrads derived from these diploids were analyzed, 4 His^+:0 His^- segregations were observed in every tetrad. Thus, the noncomplementing suppressors are linked and appear to be alleles of the *suf13* locus. A positive complementation response was observed in five of the diploids, as indicated by a His^- phenotype. After sporulation, four of these diploids failed to yield viable spores and were, therefore, not analyzed further. However, one of the diploids yielded tetrads in which the His^+:His^- phenotypes segregated 2:2, 4:0 and 3:1 in a 1:1:4 ratio. This result suggests that the diploid must have harbored two independently segregating suppressors, *suf13-1* and a new suppressor. This was confirmed by analyzing tetrads from a diploid homozygous for *his4-713* and *met2-1* and heterozygous for the putative new suppressor. Since the His and Met phenotypes cosegregated 2 His^+ Met^+:2 His^- Met^- spores per tetrad, the new suppressor, designated *suf14*, was cross-suppressing *his4-713* and *met2-1*.

To examine the ability of *suf14* to cross-suppress another type of frameshift mutation, a *his4-713 suf14* strain was crossed with a strain carrying the group II *leu2-3* mutation. The observed 2:2, 4:0 and 3:1 (Leu^+:Leu^-) segregation patterns

in tetrads derived from the diploid suggest that, like *suf13-1*, this suppressor is capable of cross-suppression. This result was confirmed in a cross homozygous for *leu2-3* and heterozygous for *suf14*, where a 2 Leu^+:2 Leu^- segregation pattern was observed in each tetrad. Thus, two suppressors were identifed in this coreversion analysis that exhibit recessive, cross-suppressing phenotypes atypical of previously identified frameshift suppressors.

Preliminary genetic mapping studies were undertaken to demonstrate that *suf13* and *suf14* represent new frameshift suppressor genes rather than alleles of previously described suppressor genes. Strains carrying *leu2-3 suf13* or *leu2-3 suf14* were crossed with a set of ten mapping strains carrying *leu2-3* and a series of 61 signal markers distributed throughout the yeast genome (see GABER and CULBERTSON 1982b). In a cross with strain A236-57B (Table 1), loose linkage was observed between *suf13* and *his3* on chromosome XV. Subsequently, *suf13* was found to map approximately 30 cM from a neighboring chromosome XV marker, *tra3* (WOLFNER *et al.* 1975, data from R. LOWENSTEIN as reported in MORTIMER and SCHILD 1980). In another cross with strain A236-57B, *suf14* was found to map approximately 40 cM from *met4*. The *met4* gene was formerly assigned to chromosome *XVII* (see MORTIMER and SCHILD 1980) but has since been shown to be located on chromosome *XIV* (KLAPHOLTZ and ESPOSITO 1982; see subsequent sections for further presentation of mapping data).

Thus, in addition to their novel cross-suppressing phenotypes, *suf13* and *suf14* appear to be new suppressor genes by virtue of their map positions. None of the previously described group II- or group III-specific suppressors map at these positions. Detailed mapping data are presented in a subsequent section.

After this initial study, 40 independent revertants of spontaneous origin were isolated from strains 852 and 853 (Table 1), which carry *his4-713*, *leu2-3*, *met2-1*, and the UAG amber nonsense mutation *trp1-1*. These revertants were chosen for analysis because they exhibited coreversion for two or more of the four auxotrophic phenotypes in these strains and were, therefore, likely to contain nongroup-specific suppressor mutations. The results of genetic studies on these corevertants are presented in Table 3.

Seventeen of the revertants were His^+ Leu^+ Met^+ Trp^- in phenotype. In 12 cases, the suppressors harbored in these corevertants were linked to the *tra3* locus on chromosome XV and are, therefore, likely to be alleles of the *suf13* locus. The remaining five corevertants were not analyzed due to poor spore viability in crosses.

One corevertant was His^+ Leu^- Met^+ Trp^- in phenotype and was subsequently shown by genetic mapping to contain an allele of the *SUF2* locus on chromosome *III*.

The remaining 22 corevertants were distinguished from those described since they were prototrophic for tryptophan. Three phenotypic classes were identified. One class, consisting of 14 corevertants, was His^+ Leu^+ Met^+ Trp^+ in phenotype. Two of these isolates were not analyzed further due to poor spore viability in crosses. A second class, consisting of three corevertants, was His^+ Leu^- Met^+ Trp^+ in phenotype. A third class, consisting of five revertants, was

TABLE 3

Distribution of independent revertants

Suppressor[a]	Media used for selection of revertants				Total
	−his	−leu	−met	−trp	
SUF2	0	0	1	0	1
SUF10	0	0	0	0	0
suf12	13	0	0	5	18
suf13	2	5	5	0	12
suf14	0	0	0	0	0
Unknown[b]	6	1	1	1	9
					—
					40

[a] Revertants were analyzed from strains 852 and 853 (Table 1) as described in the MATERIALS AND METHODS.

[b] Corevertants listed as "unknown" were not analyzed due to poor spore viability or due to loss of the suppressor phenotype in crosses.

His^+ Leu^- Met^- Trp^+ in phenotype. Two of these corevertants were not analyzed further due to poor spore viability in crosses. One representative of each of these phenotypic classes was crossed with a *his4-713 leu2-3 met2-1 trp1-1* strain. In tetrads derived from the diploids, the suppressed phenotypes cosegregated 2:2, indicating that in each cross a single mutation was responsible for suppression of the auxotrophic requirements to prototrophic phenotypes.

One of the His^+ Leu^+ Met^+ Trp^+ corevertants was crossed with a series of mapping strains containing *leu2-3*, and the segregation of the suppressor was scored relative to signal markers in the crosses. In a cross between the corevertant and strain A230-2C (Table 1), a map distance of 8 cM was observed between the suppressor and the *aro1* locus on chromosome *IV*. This approximate map location corresponds to the position assigned previously to the nongroup-specific *suf12* suppressor mutation that originally prompted this study. Thus, the corevertant probably contains an allele of the *suf12* locus.

Each of the remaining corevertants that were prototrophic for tryptophan were then crossed with a strain containing *trp1-1* and *aro1* to determine whether the suppressors exhibited linkage to *aro1*. Despite the phenotypic variation among these corevertants, all of the suppressor mutations genetically analyzed were linked to *aro1*. An attempt was made to determine whether these suppressor mutations map at the same locus. Strains carrying the suppressors were crossed in pairwise combinations to perform complementation tests and to determine whether they could undergo meiotic recombination. Although in all cases the mutations failed to complement each other, it was not possible to confirm these results by recombination analyses because all of these crosses failed to yield viable spores. Although allelism has not yet been rigorously established, the complementation results suggest that the three different phenotypic classes observed among these Trp^+ corevertants are likely to represent alleles of a single locus. As a matter of convenience, they will be referred to as alleles of the *suf12* locus.

The combined results indicate that spontaneous alleles of *suf12* and *suf13* were common among the corevertants analyzed in this study. Alleles of *SUF2* and *suf14* were rare, whereas no alleles of *SUF10* were recovered, even though *SUF10* is known to suppress *his4-713* and *met2-1*.

Genetic mapping of the suppressors

The results of tetrad analyses defining the locations of *suf12*, *suf13* and *suf14* are presented in Table 4. A genetic map showing the locations of the suppressors is presented in Figure 1. The analyses of recombinant asci that indicate the order of genes in each case are described next.

Mapping of suf12: This suppressor was located on chromosome *IV* by analyzing recombinant asci derived from a three-point cross heterozygous for *suf12*, *aro1* and *hom2* and homozygous for the suppressible *leu2-3* mutation (see cross number 555 in Table 4). Among seven tetrads that were recombinant for the *suf12-hom2* marker pair, three contained multiple crossovers, whereas the remaining four tetrads were tetratype for the *suf12-aro1* marker pair and parental ditype for the *hom2-aro1* marker pair. This result suggests that the order of genes is *aro1-hom2-suf12*. This gene order was confirmed by analyzing recombinant asci for the *suf12-aro1* marker pair. Among 17 such tetrads, three contained multiple crossovers, whereas the remaining 14 were divided into two classes. Ten tetrads were tetratype for the *aro1-hom2* marker pair and parental ditype for the *suf12-hom2* marker pair, suggesting that crossovers had occurred in the *hom2-aro1* region. Four tetrads were tetratype for the *suf12-hom2* marker pair and parental ditype for the *aro1-hom2* marker pair, suggesting that crossovers occurred in the adjacent *suf12-hom2* region.

The results suggest that *suf12* maps in the vicinity of a previously described "omnipotent" suppressor, *sup35*, which is known to cross-suppress different classes of nonsense mutations (MORTIMER and HAWTHORNE 1966; HAWTHORNE and LEUPOLD 1974; see Figure 1). Since the map distances for the *suf12-hom2* and *sup35-hom2* marker pairs are 3.5 and 4.0 cM, respectively, and since alleles of both *suf12* and *sup35* exhibit a wide range in specificity of suppression, it is conceivable that these loci are synonymous. Thus far, this hypothesis has not been rigorously tested by further recombination or complementation studies. Such studies have been hampered by technical difficulties resulting from poor sporulation of diploids derived from crosses between haploid strains carrying *suf12* and *sup35*, extremely poor viability of spores from these crosses, and the failure to identify a mutation that is suppressed by both *suf12* and *sup35* alleles.

Mapping of suf13: This suppressor was located on chromosome XV by analyzing recombinant asci from a three-point cross heterozygous for *his3*, *tra3* and *suf13* and homozygous for the suppressible *leu2-3* mutation (see cross number 528 in Table 4). Among 77 tetrads that were tetratype for the *tra3-suf13* marker pair, 26 contained multiple crossovers. Forty-six of the remaining 51 tetrads were tetratype for the *his3-suf13* marker pair and parental ditype for the *his3-tra3* marker pair. Five tetrads were reversed in phenotype, being tetratype for the *his3-tra3* marker pair and parental ditype for the *his3-suf13* marker pair. This latter ascal type is probably the result of multiple crossing over, since it is

TABLE 4

Genetic mapping of suf12, suf13 *and* suf14

Suppressor	Cross number[a]	Gene pair	PD[b]	NPD[b]	T[b]	Total	Gene pair distance (cM)
suf12	555	*suf12* × *aro1*	83	0	17	100	8.5
	555	*suf12* × *hom2*	93	0	7	100	3.5
	555	*aro1* × *hom2*	87	0	13	100	6.5
suf13	528	*his3* × *tra3*	86	1	113	200	29.8
	528	*his3* × *suf13*	43	8	147	198	49.2
	528	*tra3* × *suf13*	128	1	77	206	20.1
	557	*suf13* × *prt1*	48	11	155	214	>50
suf14	2881	*suf14* × *met4*	60	6	143	209	42.8
	2881	*suf14* × *pet2*	77	7	124	208	39.9
	2881	*met4* × *pet2*	43	22	137	202	>50
	3238	*suf14* × *met4*	55	9	118	182	47.2
	3238	*suf14* × *petx*	81	0	25	106	11.8
	3238	*met4* × *petx*	27	8	70	105	>50

[a] Genotypes of the crosses listed are as follows: 555, *leu2-3 his4-713 met2-1 trp1-1 suf12 MAT1α* × *leu2-3 trp1-1 aro1 hom2 MAT1***a**; 528, *leu2-3 his3 suf13 MAT1***a** × *leu2-3 tra3 MAT1α*; 557, *leu2-3 prt1 petx pha2 MAT1***a** × *leu2-3 his3 suf13 MAT1α*; 2881, *leu2-3 met4 suf14 MAT1***a** × *leu2-3 pet2 MAT1α*; 3238, *leu2-3 petx suf14 MAT1α* × *leu2-3 met4 MAT1***a**.

[b] PD = parental ditype; NPD = non-parental ditype; T = tetratype.

a minority class. These results suggest that the most probable gene order is *his3-tra3-suf13* (see Figure 1).

The *tra3* and *suf13* loci are separated by 20.1 cM, which places *suf13* in the vicinity of *cpa1*, a mutation that confers arginine auxotrophy in the presence of excess uracil (data of F. HILGER, as reported in MORTIMER and SCHILD 1980). The gene order for *suf13* and *cpa1* relative to other linked markers has not been established. These markers are, therefore, shown within parentheses on the genetic map. Since *met7* has been shown to be located between *his3* and *tra3* (data of R. LOWENSTEIN, as reported in MORTIMER and SCHILD 1980), the most probable gene order for this chromosome region is *his3-met7-tra3-(suf13-cpa1)*.

The mutation *prt1* has been shown by mitotic mapping to be located on chromosome XV (MORTIMER and HAWTHORNE 1973). Cross number 557 (Table 4) shows that *suf13* and *prt1* are loosely linked. Although the map distance calculated by the standard formula of PERKINS (1949) is slightly greater than 50 cM, a chi-square value of 22.39 indicates that the deviation in the data from that expected for complete nonlinkage between the two markers is significant. This result and other recombination tests described in MORTIMER and SCHILD (1980) suggest the likelihood that the gene order is *his3-met7-tra3-(suf13-cpa1)-prt1*.

Mapping of suf14: This suppressor was located on chromosome XIV by analyzing tetrads from two separate three-point crosses. The first cross (number 2881 in Table 4) was heterozygous for *pet2*, *suf13* and *met4* and homozygous for the suppressible *leu2-3* mutation. Data from this cross indicate that *suf14* is located 42.8 cM from *met4* and 39.9 cM from *pet2*. Due to an excessive number of double crossovers, the standard method for determining gene order was not

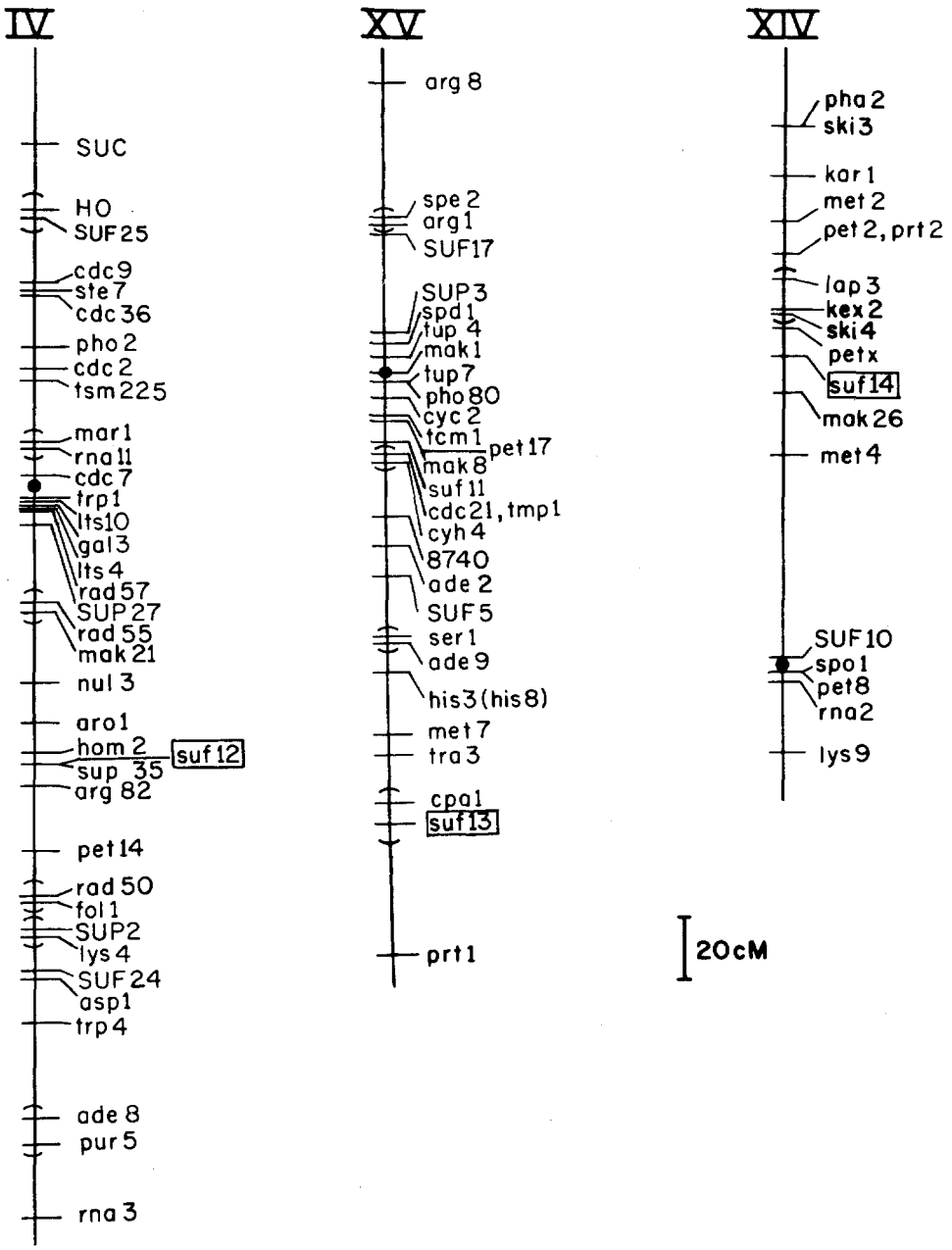

FIGURE 1.—Genetic map of three yeast chromosomes showing the locations of *suf12*, *suf13* and *suf14*. The genetic map for chromosomes *IV* and XV is drawn as shown in MORTIMER and SCHILD (1980). The genetic map for chromosome *XIV* has been revised from that shown in MORTIMER and SCHILD (1980) and is based on data from this communication and more extensive mapping data from KLAPHOLTZ and ESPOSITO (1982). The revised map includes markers formerly placed on chromosome *XVII* which are now known to reside on chromosome *XIV*. Black circles indicate the locations of the centromeres. Dotted lines represent linkages established by trisomic segregation analysis. When the orientation of two or more genes relative to outside markers is in question, these genes have been enclosed within parentheses.

used. However, the genetic distances between markers were suggestive of the correct order. Since *suf14* is approximately equidistant from *met4* and *pet2* and since *met4* and *pet2* are virtually unlinked (greater than 50 cM), the most likely gene order is *pet2-suf14-met4*. Furthermore, in separate crosses each of these three markers failed to exhibit linkage to a centromere. They must, therefore, comprise a single linkage group on the same chromosome arm.

The second three-point cross (number 3238 in Table 4) was heterozygous for *petx*, *suf14* and *met4*. The *petx* mutation, which maps between *met4* and *pet2*, is 11.8 cM from *suf14*. The order of genes was determined by analyzing tetratype asci for the *petx-suf14* marker pair. Among 25 such asci, 12 contained double crossovers. Ten of the remaining 13 asci were tetratype for the *petx-met4* marker pair and parental ditype for the *suf14-met4* marker pair. The other three asci were reversed in phenotype, being tetratype for the *suf14-met4* marker pair and parental ditype for the *petx-met4* marker pair. These three tetrads are probably the result of double crossing-over since they represent a minority ascal class. By combining the results of both crosses, the most probable order is *pet2-petx-suf14-met4*.

The complete genetic map for chromosome *XIV* showing the location of *suf14* is provided in Figure 1. This genetic map differs from previously published maps of this chromosome (see MORTIMER and SCHILD 1980). The left arm of the chromosome is partially comprised of markers formerly thought to reside on chromosome *XVII*. The extensive genetic data of KLAPHOLTZ and ESPOSITO (1982) provide ample justification for the revised chromosome *XIV* map shown in the figure.

Spectrum of suppression

Since preliminary results indicated that alleles of *suf12*, *suf13* and *suf14* are capable of cross-suppressing different types of mutations, the spectrum of suppression for a representative allele of each locus was tested in greater detail (Table 5).

Although alleles of *suf12* proved difficult to examine due to poor spore viability in crosses, the *suf12-2* allele has been shown to suppress *leu2-3* (a group II frameshift mutation suppressed by glycine tRNA encoding suppressors), *his4-713* (a group III frameshift mutation suppressed by proline tRNA encoding suppressors), *met2-1* (a mutation that is cross-suppressible by glycine and proline tRNA encoding suppressors), *trp1-1* (a UAG nonsense mutation) and *leu2-2* (a UGA nonsense mutation). This suppressor does not suppress all mutations regardless of type, as evidenced by its failure to suppress *his4-506* (a group I frameshift mutation), *his4-519* (a group II frameshift mutation), *ade2-1* and *lys-1* (UAA nonsense mutations). Thus, *suf12-2* exhibits an allele-specific pattern of suppression, but the mutations that are suppressed need not be related to each other according to mutational type.

The *suf13-1* and *suf14-1* alleles fail to suppress any of the nonsense mutations listed in Table 5. However, they do suppress certain frameshift mutations of group I, group II and group III. Thus, these suppressors differ in phenotype from *suf12-2* but fit within the broad definition of nongroup-specific suppression by virtue of their ability to suppress different types of frameshift mutations.

TABLE 5

Cross-suppression of frameshift and nonsense mutations by nongroup-specific suppressors

Allele	*his4-506*	*his4-520*	*his4-208*	*his4-707*	*his4-717*	*his4-507*	*his4-518*	*his4-206*	*his4-38*	*his4-519*	*his4-504*	*his4-211*	*leu2-3*	*arg4-20*	*his4-713*	*his4-712*	*met2-1*	*ade2-1*	*arg4-17*	*lys1-1*	*his4-644*	*leu2-1*	*met8-1*	*his4-580*	*trp1-1*	*leu2-2*	*his4-260*
Type of mutation[a]	I							II							III		II + III	UAA					UAG			UGA	
Suppressor																											
suf12-2	–	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	–	[b]	[b]	+	[b]	+	–	+	–	[b]	–	[b]	[b]	[b]	[b]	+	+	[b]
suf13-1	–	+	–	–	–	+	–	–	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–
suf14-1	–	+	–	–	–	+	–	–	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–

[a] The roman numerals I, II, and III refer to groups of ICR-induced frameshift mutations (CULBERTSON *et al.* 1977). UAA, UAG, and UGA refer to ochre, amber, and opal nonsense mutations, respectively.

[b] Suppression of these alleles by *suf12-1* was not tested due to poor spore viability in crosses.

DISCUSSION

Prior to this study, two major classes of extragenic suppressors had been identified among revertants of *his4* frameshift mutations in *Saccharomyces cerevisiae* (CULBERTSON *et al.* 1977; CULBERTSON, UNDERBRINK and FINK 1980; CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b). Molecular studies have shown that representative suppressors of these two classes encode altered glycine and proline tRNAs containing base insertions in the anticodon loops (CUMMINS, DONAHUE and CULBERTSON 1982; GABER and CULBERTSON 1982a). These suppressors exhibit specific patterns of suppression that are explicable on the basis of codon-anticodon interactions leading to quadruplet rather than triplet reading of the genetic code.

This communication describes the isolation and genetic properties of recessive frameshift suppressors mapping at three loci. These suppressors exhibit behavior atypical of the tRNA-encoding group-specific suppressor genes. Representative alleles of these suppressor genes have been shown to suppress +1 frameshift mutations that have been classified into different groups according to the types of codons containing the base insertions. In some cases certain nonsense mutations are also suppressed. These suppressors have, therefore, been designated as nongroup-specific in phenotype. The distinguishing features of these suppressors imply the existence in yeast of molecular mechanisms for frameshift suppression involving components of translation other than glycine and proline tRNAs.

Nongroup-specific suppressors were identified in these studies by determining whether extragenic revertants of one type of frameshift mutation ever exhibit simultaneous coreversion for other types of frameshift mutations or for nonsense mutations. Corevertants exhibiting a variety of cross-suppressing phenotypes were isolated in strains carrying a group II frameshift mutation (*leu2-3*), a group III frameshift mutation (*his4-713*), a UAG amber nonsense mutation (*trp1-1*) and the ICR-induced *met2-1* mutation described in this study.

The *met2-1* mutation is interesting in that it is cross-suppressed by group-specific suppressor mutations in genes known to encode altered glycine and proline tRNAs. One of several plausible mechanisms may be responsible for the unusual properties of the *met2-1* allele. The group-specific suppressors may interact with glycine and/or proline codons in the vicinity of the *met2-1* mutation rather than with the codon containing the mutation itself. This could occur particularly if the affected region of the protein is not essential for catalytic activity. Alternatively, *met2-1* may represent a +1 G:C insertion in adjacent glycine and proline codons within the *met2* gene, such that either glycine or proline suppressor tRNAs could act at the site of mutation by reading overlapping sets of four bases in the mRNA to generate a normal amino acid sequence. The *met2-1* mutation proved useful in these studies since it is suppressed by the nongroup-specific suppressors as well.

Corevertants exhibiting nongroup-specific suppression patterns map at three loci, designated *suf12*, *suf13* and *suf14*. The *suf12* locus maps 3.5 cM from *aro1* on chromosome *IV*. The *suf13* locus maps 20.1 cM from *tra3* on chromosome XV. In the course of mapping the *suf14* locus, results were obtained that

substantiate the proposal of KLAPHOLTZ and ESPOSITO (1982) for revision of the chromosome *XIV* map and elimination of chromosome *XVII* from the genetic map. The *suf14* suppressor is linked to two markers that were formerly thought to reside on chromosome *XVII*, *pet2* and *met4* (MORTIMER and SCHILD 1980). The suppressor is also limited to *petx*, which was placed on chromosome *XIV* by trisomic segregation analysis (WICKNER and LEIBOWITZ 1976; WICKNER 1979). The combined chromosome map (Figure 1), which includes chromosome *XIV* and the former chromosome *XVII* linkage groups, is supported by trisomic analysis, standard meiotic linkage, and by the observation that markers assigned in previous studies to these two chromosomes cosegregate in the absence of genetic recombination in strains homozygous for the rec$^-$ mutant *spo11-1* (KLAPHOLTZ and ESPOSITO 1982). Our results are consistent with those of KLAPHOLTZ and ESPOSITO (1982) and support the contention that yeast contains 16 rather than 17 genetically defined chromosomes.

Tetrad data from genetic mapping studies suggest that *suf12* may be located at the same position as the "omnipotent" suppressor *sup35* (HAWTHORNE and MORTIMER 1968). Alleles of *sup35* exhibit phenotypic variation and are known to cross-suppress certain nonsense mutations belonging to different classes (HAWTHORNE and LEUPOLD 1974). Since alleles of *suf12* also exhibit phenotypic variation and cross-suppress several types of frameshift and nonsense mutations, they bear a striking phenotypic resemblance to *sup35* alleles. Although the deleterious effects of these suppressors on sporulation and spore viability have hampered our ability to complete rigorous complementation and recombination tests, the phenotypic similarity of these suppressors and their close proximity on the genetic map suggest the possibility that *sup35* and *suf12* may be synonymous.

The behavior of *suf12* and *sup35* alleles is atypical of mutations in genes that encode suppressor tRNAs. Since several lines of evidence implicate *sup35* as a gene that possibly encodes a ribosomal protein, mutations at this locus may resemble the ribosomal ambiguity mutations in bacteria that cause generalized misreading of the genetic code (GORINI 1974; LIEBMAN and CAVENAGH 1979; LIEBMAN, CAVENAGH and BENNETT 1980; LIEBMAN and CAVENAGH 1981). However, definitive identification of the *sup35* gene product is still lacking. In addition, the *suf12* locus could represent an adjacent rather than a synonymous gene. It should be possible to distinguish between these possibilities using molecular cloning techniques.

The *suf13* and *suf14* genes do not map at positions previously assigned to omnipotent suppressors, and alleles of these loci fail to suppress nonsense mutations. These suppressors are, however, nongroup-specific in phenotype since they suppress certain frameshift mutations of groups I, II and III. Representative mutations in each of these groups have been shown to be +1 G:C insertions, but each of the groups differ in the types of codons containing the base insertions (DONAHUE, FARABAUGH and FINK 1981). Mutations in different groups would not be expected to be suppressed by informational suppressors encoding specific tRNAs that interact directly at the sites of the frameshift mutations. Since suppressor genes encoding glycine and proline tRNAs are known to be specific for suppression of +1 G:C insertions in glycine (group II)

and proline (group III) codons, the nongroup-specific *suf13* and *suf14* suppressors are unlikely candidates for genes encoding these tRNAs.

Suppressor mutations mapping at or near the *suf13* and *suf14* loci have also been identified among corevertants of the group II *his4-206* and *leu2-3* frameshift mutations (GABER and CULBERTSON 1982b). These alleles have also been shown to suppress group I frameshift mutations and are, therefore, nongroup-specific in phenotype. However, the *his4-206* mutation is not suppressed by the *suf13* or *suf14* alleles isolated in the present study. Thus, *suf13* and *suf14* may resemble *suf12* and *sup35* in that different alleles of these loci suppress different mutations. Alternatively, phenotypically distinguishable suppressors mapping in the same chromosome region may represent mutations in distinct but closely linked genes such that they affect the functions of different gene products or activities.

Although the *his4* frameshift mutations suppressed by *suf13* and *suf14* do not belong to the same group, they may be related according to their map position within the *his4* coding region. Three of the four *suf13*- and *suf14*-suppressible mutations, including *his4-520*, *his4-507 and his4-504*, are clustered in a small region near the 3′ end of *his4A* (CULBERTSON *et al.* 1977). The only exception, *his4-713*, maps close to the 3′ end of the coding region in *his4C* (DONAHUE, FARABAUGH and FINK 1981). More extensive genetic analysis will be required to determine whether the ability to be suppressed by these nongroup-specific suppressors bears any relationship to map position within the *his4* gene. The existence of such a relationship would be an important consideration in elucidating possible mechanisms of suppression.

Several molecular mechanisms for *suf13*- and *suf14*-mediated suppression of frameshift mutations are possible. At the translational level, altered tRNA base modification enzymes might exhibit pleiotropic effects on multiple families of tRNAs and cause mistranslation and suppression of different types of +1 frameshift mutations. A similar model has been proposed as one possible mechanism of suppression for recessive frameshift suppressors in *Salmonella* (RYASATY and ATKINS 1968; RIDDLE and ROTH 1972).

Alternatively, *suf13* or *suf14* might encode tRNAs other than the glycine and proline isoacceptors which contain four base anticodons. Such suppressing tRNAs might interact at sites in the mRNA in regions neighboring the sites of mutation rather than at the sites of the mutations themselves. Suppression would result from restoration of the reading frame if the region between the site of mRNA:tRNA interaction and the site of mutation is not essential for catalytic activity of the enzyme, as is the case for certain *sufJ* suppressible frameshift mutations in the *hisD* gene in *Salmonella* (BOSSI and ROTH 1981). Although genetic dominance of the suppressors would be predicted from this model, it is possible that the recessive phenotypes of the nongroup-specific suppressors may simply reflect a low efficiency of suppression.

Other translational components, such as ribosomal proteins or any of the various translational factors, might be altered in such a way as to cause suppression of certain frameshift mutations by altering the phase fidelity of translation, resulting in a shift to the normal phase and production of a functional protein.

One precedent for a more exotic mechanism of nongroup-specific suppression

is based on the existence of suppressor mutations mapping at the *lon* locus in *Escherichia coli* (see MOUNT 1980). The *lon* gene has been shown to affect the activity of a protease that degrades abnormal amino acid sequences. The suppressor mutations mapping within this gene exhibit genetic properties resembling to some extent those of the nongroup-specific suppressors in yeast. This model requires an *in vivo* mechanism for spontaneous low level reading frame slippage similar to that described by ATKINS *et al* (1979). If such reading frame slippage occurs in yeast, it is possible that the gene products of nongroup-specific suppressor loci might act posttranslationally by conferring stability and allowing accumulation of an altered but functional gene product which would otherwise undergo proteolytic degradation. This model also requires that the incorrect stretch of amino acids between the point of the frameshift mutation and the site of reading frame slippage does not abolish catalytic activity.

Further genetic and molecular characterization of the suppressor genes and gene products will be required in order to elucidate the mechanisms of nongroup-specific frameshift suppression since, at present, the available information on these suppressors is insufficient to distinguish between various possible molecular models.

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