FRAMESHIFT SUPPRESSION IN *SACCHAROMYCES CEREVISIAE*. II. GENETIC PROPERTIES OF GROUP II SUPPRESSORS

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ABSTRACT

Suppressors of ICR-induced mutations that exhibit behavior similar to bacterial frameshift suppressors have been identified in the yeast *Saccharomyces cerevisiae*. The yeast suppressors have been divided into two groups. One of these groups (Group II: *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*) appears to include a set of informational suppressors in which the vehicle of suppression is glycyl-tRNA. Some of the genetic properties of Group II suppressors are described in this communication.——Corevertants of the Group II frameshift mutations *his4–519* and *leu2–3* have been characterized to determine the spectrum of reversion events induced by the frameshift mutagen ICR-170. Seventy-three ICR-induced corevertants were analyzed. With the exception of one corevertant, which carried an allele of *SUF1*, all carried alleles of *SUF3* or *SUF5*. *SUF1*, *SUF3*, *SUF4* and *SUF6* were represented among spontaneous and UV-induced corevertants. In the course of these experiments one of the suppressors was mapped. *SUF5*, the probable structural gene for $tRNA^{GLY1}$, is located between *ade2* and *ade9* on chromosome *XV*.——*SUF1*, *SUF4* and *SUF6* have novel properties and comprise a distinct subset of suppressors. Although these suppressors show no genetic linkage to each other, they share several common features including lethality in haploid pairwise combinations, reduced $tRNA^{GLY3}$ isoacceptor activity and increased efficiency of suppression in strains carrying the cytoplasmically inherited [PSI] element. In addition, strains carrying *SUF1*, *SUF4* or *SUF6* are phenotypically unstable and give rise to mitotic Suf+ segregants at high frequency. These segregants invariably contain a linked, second-site mutation that maps in or adjacent to the suppressor gene itself. Strains carrying any of these suppressors also give rise to mitotic segregants that exhibit enhanced efficiency of suppression; mutations responsible for this phenotype map at two loci, *upf1* and *upf2*. These genes show no genetic linkage to any of the Group II suppressors.——Methods that permit positive selection for mutants with decreased or enhanced efficiency of suppression have been devised in order to examine large numbers of variants. The importance of these interacting mutants is underscored by their potential utility in studying suppressor function at the molecular level.

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MUTATIONS induced by the acridine half-mustards (ICR compounds) have been studied extensively in *Salmonella typhimurium* (AMES and WHITFIELD 1966; YOURNO and HEATH 1969; YOURNO 1971). A large proportion of the mutants contain +1 G/C insertions in monotonous runs of G/C base pairs. These types of mutations shift the reading frame of the message out of phase beyond the point of insertion and result in the production of a nonfunctional protein.

ICR-induced revertants of +1 G/C insertions in the Salmonella histidine operon frequently carry mutations mapping at sites external to the operon that confer a His^+ phenotype (RIDDLE and ROTH 1970). These external suppressors map at sites on the bacterial chromosome known to contain tRNA genes, and altered forms of tRNA have been shown to mediate framesehift suppression by reading a four-base codon. Strains of Salmonella carrying the frameshift suppressor *sufD* produce a glycyl-tRNA with the nucleotide quadruplet CCCC at the anticodon position, instead of CCC normally found in wild type (RIDDLE and ROTH 1972a,b; RIDDLE and CARBON 1973). The addition of this extra base is presumed to permit recognition of the four-base code word GGGN and thereby correct the reading frame. A second class of frameshift suppressors was shown to alter the chromatographic behavior of prolyl-tRNA (RIDDLE and ROTH 1972b). These suppressors are also believed to act by reading a four-base code word. These results demonstrate that the acridine half-mustards derive their powerful mutagenic activity in part from an ability to promote G/C base pair insertions in DNA.

Two groups of external suppressor mutations, Group II and Group III, have been identified among revertants of ICR-induced mutations at the *his4* locus in *Saccharomyces cerevisiae* (CULBERTSON *et al.* 1977). Their properties suggest that they may be analogous to bacterial frameshift suppressors. Elution profiles obtained by co-chromatography of tRNA from wild-type strains and strains carrying the suppressors suggest that one group (Group II: *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*) may be mutations in the structural genes for glycyl-tRNAs. Some of the genetic properties of these suppressors are described in this communication.

Corevertants of the Group II frameshift mutations *his4–519* and *leu2–3* have been characterized to determine the spectrum of reversion events induced by the frameshift mutagen ICR-170. ICR-induced reversion of these mutations results in suppressors that map at the *SUF3* and *SUF5* loci. By contrast, spontaneous and UV-induced revertants carry mutations that map at *SUF1*, *SUF3*, *SUF4* and *SUF6*. Similar results were obtained in a genetic study of Group III suppressors (CUMMINS *et al.* 1980). ICR-induced revertants of the Group III mutation *his4–713* were shown to carry alleles of the *SUF2* locus; whereas, spontaneous and UV-induced revertants carry mutations that are distributed among six Group III suppressor loci. These studies demonstrate that only three of the 11 suppressor genes obtained by reversion of ICR-induced *his4* mutations are targets for mutagenesis by ICR-170.

Strains carrying the Group II suppressor, *SUF5*, have been shown to produce a chromatographically altered species of $tRNA^{GLY1}$, suggesting that *SUF5* is the structural gene for this tRNA (CULBERTSON *et al.* 1977). In the course of this study, *SUF5* was mapped on chromosome *XV* between *ade2* and *ade9*.

Three of the five Group II suppressors, *SUF1*, *SUF4* and *SUF6*, result in reduced isoacceptor activity of $tRNA^{GLY3}$ and have unusual properties that are described in this communication. The genetic map positions of these suppressors have not yet been determined, but pairwise crosses show that these suppressors represent three unlinked genes. *SUF1*, *SUF4* and *SUF6* are lethal in combination with each other; double-mutant spores carrying two suppressors cannot be recovered after meiosis. In addition, it has been shown that the efficiency of suppression by *SUF1*, *SUF4* or *SUF6* is increased in strains carrying the cytoplasmically inherited [PSI] element (CULBERTSON *et al.* 1977). [PSI] also increases the efficiency of the serine-inserting ochre suppressors *SUP17* and *SUQ5* (*SUQ5* ≡ *SUP15* ≡ *SUP16*), and autonomously suppresses certain ochre mutations (*e.g.*, *trp5–48*) (COX 1965; LIEBMAN, STEWART and SHERMAN 1975; LIEBMAN and SHERMAN 1979; ONO, STEWART and SHERMAN 1979).

In this study we show that strains carrying *SUF1*, *SUF4* or *SUF6* are phenotypically unstable and give rise to mitotic Suf^+ segregants at high frequency. These segregants invariably contain a mutation that maps in or adjacent to the suppressor gene itself. Mitotic and meiotic recombination analysis shows that they are second-site mutations, rather than back-mutations to wild type. A method is described in which second-site revertants of the suppressors can be isolated by positive selection for canavanine resistance in cells carrying a suppressor and a Group II suppressible *can1* mutation.

In addition, mutations conferring enhanced efficiency of framesehift suppression have been isolated as mitotic segregants from strains carrying *SUF1*, *SUF4* or *SUF6*. These mutations, designated *upf* for "up-frameshift suppressor," can be recovered from strains carrying a suppressor and the suppressible *his4* allele *his4–38*. These strains are temperature sensitive for growth on minimal medium. Up-suppressor mutations confer the ability to grow at the restrictive temperature. Mutations of this type map at two loci that show no genetic linkage to each other or to the Group II suppressor loci. Unlike the [PSI] element, which also confers growth at the restrictive temperature in strains carrying *his4–38* and *SUF1*, *SUF4* or *SUF6*, *upf* mutations are chromosomally inherited. The molecular basis of temperature-sensitive growth associated with suppression of *his4–38* is not yet understood. Two models consistent with the observations are discussed in relation to the interaction between frameshift suppressors and *upf* mutations.

The similarities of *SUF1*, *SUF4* and *SUF6* suggest that these suppressors may represent redundant forms of the same gene. We anticipate that the peculiar genetic properties of these suppressors will eventually be resolved at the molecular level and will provide information on the synthesis and function of the suppressing tRNA.

MATERIALS AND METHODS

Yeast strains and genetic methods: All strains used in this study are derivatives of the wild-type laboratory strain S288Cα. The isolation of frameshift mutations, frameshift suppressors and the characterization of strains used in this study have been described (CULBERTSON *et al.* 1977). Genetic methods and nomenclature are those described in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and LAWRENCE 1971).

Media: The following types of media were used: YEPD, which contains 2% Bactopeptone, 1% yeast extract, 2% glucose and 2% agar; minimal medium, which contains 6.7 g/l Difco Yeast Nitrogen Base, 2% glucose and 2% agar; KAC (sporulation medium), which contains 1% potassium acetate, 0.1% glucose, 1.25 g/l yeast extract and 2% agar. When required, purines, pyrimidines, or amino acids were added to minimal medium at concentrations given in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and LAWRENCE 1971).

Coreversion of frameshift mutation: His+ Leu+ corevertants of a strain carrying the frameshift mutations *his4–519* and *leu2–3* were isolated as follows: Single colonies were isolated on YEPD plates. Cells from individual colonies were picked and 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. In this procedure, the cloning of independent lines prior to mutagenesis insured that mutants obtained from different culture tubes were of independent origin. The cells were centrifuged, washed twice with sterile water and concentrated 10-fold by resuspension in 0.3 ml of water. 0.1 ml aliquots were spread on minimal plus leucine plates to select for His+ revertants. Plates were either incubated without mutagenesis, irradiated with UV for 25 sec at a dose that gave 80% survival, or treated with ICR-170 (2-methoxy-6-chloro-9-[-3-(ethyl-2-chloroethyl) aminopropylamino]acridine·2HCl (Polysciences, Inc.). ICR-170 mutagenesis was performed according to the method of CULBERTSON *et al.* (1977) with the following modification: cells were mutagenized directly on minimal plus leucine plates containing 0.2 ml YEPD. The small amount of YEPD added to the synthetic medium is insufficient to supplement the His and Leu auxotrophies, but is absolutely required for the mutagenic activity of ICR-170. The reason for the YEPD requirement is unknown. Following the appearance of His+ revertants, the cells were replica-plated to minimal medium to detect His+ Leu+ corevertants. The corevertants were analyzed in genetic crosses described in RESULTS.

Genetic mapping: Linkage of *SUF5* to markers on chromosome *XV* was determined in standard crosses, and linkage distances were calculated in centimorgans (CM) using the equation X (CM) $= 50$ [tetratype asci $+ 6$(nonparental-ditype asci)]/total asci (PERKINS 1949). The *ade9* mutation used in this mapping study was identified in a collection of ICR-170-induced auxotrophs and is not suppressed by *SUF5* (GABER, EDELMAN and CULBERTSON, unpublished).

Isolation of suppressible canavanine-resistant mutants: Mutations conferring resistance to canavanine were induced by ICR-170. Ten μl of a 1 mg/ml solution of ICR-170 was placed on a YEPD plate that had first been covered with a lawn of canavanine-sensitive cells. The strain to be mutagenized carried the group II mutations *his4–519* and *leu2–3* so that canavanine-resistant mutants could be analyzed in a coreversion test. Cells were grown in the presence of ICR-170 overnight at 30° on YEPD medium and then replica-plated to minimal medium supplemented with histidine, leucinine and 60 mg/l L-canavanine sulfate (Sigma). The plates were incubated for 5 days. Colonies forming a ring around the drop of ICR-170 were picked, purified and tested as described in RESULTS.

Estimation of cell-doubling time: The doubling time of a strain carrying *SUF1* was compared to that of a Suf+ revertant of this suppressor in order to determine whether the apparent high frequency of reversion observed for *SUF1* is due to a selective growth advantage of Suf+ segregants. A strain carrying *his4–519 leu2–3 can1–101 SUF1* was plated on media containing canavanine to select for Suf+ revertants. The *can1–101* mutation is suppressible by *SUF1* (see RESULTS). Thus, the *his4–519 leu2–3 can1–101 SUF1* strain is canavanine sensitive due to suppression, whereas Suf+ revertants of this strain are canavanine resistant due to loss of suppressor function. Suf+ revertants selected in this way were purified and tested for loss of suppressor function by replica-plating to minimal media lacking histidine and leucine. A His$^-$ Leu$^-$ Can^r

revertant was used to compare the doubling time of the revertant with the isogenic *SUF1* parental strain from which it was derived.

Cultures of the two strains were prepared by inoculating YEPD medium at a starting density of 1×10^6 cells per ml. The *SUF1* strain was precultured in minimal medium to ensure that 100% of the cells in the primary culture initially carried the suppressor. The Suf+ strain was precultured in minimal medium supplemented with histidine and leucine. The cultures were monitored at 2 hr intervals by diluting and plating aliquots on YEPD medium to determine the number of viable cells per ml. It was necessary to test the *SUF1* culture at each time point for the appearance of Suf+ revertants by comparing the plating efficiency of the culture on YEPD medium and minimal medium. In addition, colonies that grew on YEPD medium were replica-plated to minimal medium, and any colony that failed to grow on minimal medium was scored as a Suf+ revertant. This procedure permitted a determination of the percentage of cells at each time point that had maintained a functional suppressor. The doubling time of the SUF1 strain was calculated using a culture in which more than 99% of the cells had retained the *SUF1* phenotype at the end of the logarithmic phase of growth. The doubling times of the *SUF1* and Suf+ strains could then be accurately compared by plotting log cell concentration *vs.* time of growth.

As an additional control, a reconstitution experiment was performed in which *SUF1* cells were mixed with Suf+ revertant cells at a ratio of 4 to 1, and the ratio of the 2 types of cells was monitored throughout the growth of the culture, using the methods described above.

RESULTS

The results are divided into two parts. The first part describes the distribution of external suppressors obtained by reversion of Group II frameshift mutations at the *his4* locus. Mapping data for one of the ICR-induced suppressors are also presented. The second part describes methods for isolating and analyzing mutations that affect the efficiency of frameshift suppression.

Analysis of frameshift revertants

Coreversion of Group II frameshift mutations: Table 1 shows the distribution of suppressor mutations identified among corevertants in a strain carrying the ICR-induced group II mutations, *his4–519* and *leu2–3*. His+ Leu+ corevertants were analyzed by linkage analysis to determine whether they carried the

TABLE 1

Analysis of his4–519 leu2–3 *corevertants*

Method of induction	Number of independent isolates					Total corevertants analyzed	Reversion frequency*
	SUF1	*SUF3*	*SUF4*	*SUF5*	*SUF6*		
ICR-170	1	56	0	16	0	73	
Spontaneous	0	1	1	0	1	3	1
UV-irradiation	2	0	0	0	0	2	14

* Spontaneous reversion frequencies are expressed as the number of revertants/10^8 viable cells plated. UV-induced reversion frequencies are expressed as the number of revertants/10^8 survivors obtained folloing a 25 sec exposure to UV at a dose of 800 ergs/cm^2/sec. The survival rate following UV exposure with this strain was 80%. The method used for ICR-170 mutagenesis in which the mutagen is spotted directly on a petri plate containing a lawn of cells does not permit precise calculation of reversion frequency in response to a given dose. However, we estimate that the frequency of ICR-170-induced revertants among survivors of mutagenesis is in excess of 100-fold above the spontaneous reversion frequency of *his4–519*.

previously identified suppressors (*SUF1*, *SUF3*, *SUF4*, *SUF5* or *SUF6*) or new suppressor mutations. Each corevertant was crossed with a set of strains carrying *his4–519*, *leu2–3* and each of the five Group II suppressors. A corevertant was classified as carrying one of the known group II suppressors if the cross involving that suppressor failed to segregate His$^-$ Leu$^-$ spores in a sample of 24 tetrads.

ICR-induced corevertants predominantly carry alleles of the *SUF3* locus (56/73) and the *SUF5* locus (16/73). One ICR-induced corevertant carried an allele of the *SUF1* locus. Since the *SUF1* allele was a rare isolate among ICR-induced suppressors, it is possible that it was not induced by the mutagen, but was of spontaneous origin. Most of the spontaneous and UV-induced His$^+$ revertants of *his4–519* had a Leu$^-$ phenotype and were not analyzed further. As a result of this difficulty, very few spontaneous or UV-induced corevertants have been analyzed. The three spontaneous corevertants that were obtained mapped at the *SUF3*, *SUF4* and *SUF6* loci. Two UV-induced corevertants were both alleles of the *SUF1* locus. No new suppressors were identified in this coreversion analysis.

Mapping of SUF5 *on chromosome* XV: *SUF5* was originally mapped on chromosome *XV* in a cross that was homozygous for *his4–519*, heterozygous for the suppressor and heterozygous for *ade2*. Preliminary data suggested that *SUF5* and *ade2* were approximately 13 cM apart. Subsequently, *SUF5* was mapped more precisely in a four-point cross involving the chromosome *XV* markers, *ade2*, *ade9* and *his3*, and the suppressor (Table 2; Figure 1). Since *his3* was heterozygous in the cross, it was not convenient to follow the segregation of the suppressor by suppression of *his4–519*. Instead, the diploid was homozygous for the suppressible marker *leu2–3* and the segregation of the suppressor was scored on leucineless medium.

The mapping data presented in Table 2 show that *ade2* and *SUF5* are separated by 12 cM. Tetratype asci for the *ade2-SUF5* marker pair were analyzed to determine the order of the two genes with respect to *ade9* and *his3*. Among 34 tetratype asci, 18 had the parental configuration for the *ade9-SUF5* marker pair and the tetratype configuration for the *ade2-ade9* marker pair. Six tetrads had

TABLE 2

Mapping of SUF5 *on chromosome* XV

Marker pair*	PD	NPD	T	Total	Gene pair distance (cM)
his3–ade2	21	7	107	135	>50
his3–ade9	100	0	36	136	13
his3–SUF5	35	2	100	137	41
ade2–ade9	37	6	99	142	48
ade2–SUF5	106	0	34	140	12
ade9–SUF5	50	1	89	140	34

* The cross was *leu2–3 ade9 lys2 suf+* × *leu2–3 his3 ade2 SUF5*. The segregation of the suppressor was scored by its ability to suppress *leu2–3*.
PD = parental ditype. NPD = nonparental ditype. T = tetratype.

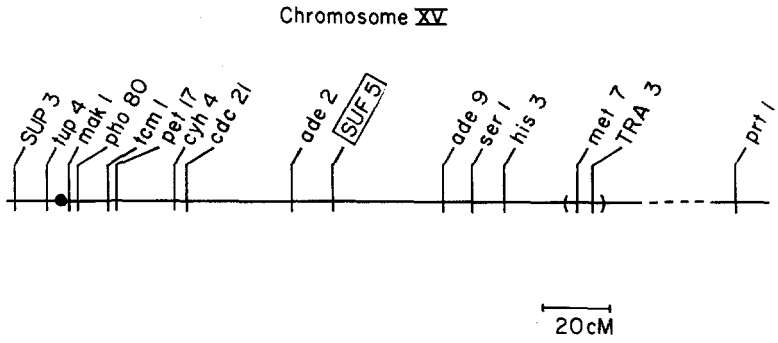

FIGURE 1.—The location of *SUF5* is shown on the genetic map of chromosome *XV*.

the tetratype configuration for the *ade9-SUF5* pair and the parental configuration for the *ade2-ade9* pair. These results suggest that the probable gene order is *ade2-SUF5-ade9-his3*. A map of chromosome *XV* showing the location of *SUF5* is presented in Figure 1.

Analysis of mutations affecting suppressor efficiency

Isolation of suppressible canavanine-resistant mutants: The phenotypic stability of Group II suppressors was examined through the use of suppressible canavanine-resistant mutants isolated by the procedure described in MATERIALS and METHODS. A preliminary coreversion test suggested that suppressible frameshift mutations were represented frequently among ICR-170-induced canavanine-resistant mutants. The coreversion test operates on the principle that ICR-170-induced His$^+$ revertants of a strain carrying *his4–519* and *leu2–3* exhibit concommitant reversion to a Leu$^+$ phenotype through the induction of external frameshift suppressors capable of suppressing both frameshift markers. As described above, most of the ICR-induced corevertants carry alleles of *SUF3* or *SUF5*.

Since the canavanine-resistant mutations were isolated in a strain carrying *his4–519* and *leu2–3*, it was possible to test for suppression of the canavanine-resistant phenotype by reverting *his4–519 leu2–3 can1* strains to a His$^+$ phenotype with ICR-170 and checking for coreversion to a Leu$^+$ Can^s phenotype. Canavanine-resistant mutants that exhibited a Leu$^+$ Can^s phenotype in this test were classified as putative suppressible frameshift mutants.

One canavanine-resistant mutant, designated *can1–101*, was analyzed in detail in crosses with strains carrying *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*. Formally, these crosses were of the type *his4–519 leu2–3 can1–101 suf*$^+$ × *his4–519 leu2–3 CAN1*$^+$ *SUFX*. 2:2, 0:4 and 1:3 (Can^r:Can^s) segregation patterns observed in a 1:1:4 ratio in tetrads from these crosses were taken as evidence for suppression of the *can1–101* allele by an unlinked frameshift suppressor. Crosses heterozygous for *SUF1*, *SUF3*, *SUF4*, *SUF5* or *SUF6* gave the expected segregation pattern. Furthermore, all canavanine-resistant spores from these crosses were phenotypically Suf$^+$ (*i.e.*, His$^-$) as would be expected if the observed deviation from a normal 2:2 segregation was the result of suppression of *can1–101*. In

another set of crosses heterozygous for each suppressor and homozygous for *can1–101*, a 2:2 segregation of resistance to sensitivity was observed. All resistant spores were phenotypically Suf$^+$ and all sensitive spores carried a suppressor. These crosses define *can1–101* as a group II frameshift mutation that is suppressed by *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*.

Revertants of the suppressors: The suppressible mutation *can1–101* was used to develop a convenient method for isolating revertants for Group II suppressors. The scheme for positive selection of suppressor revertants is based on the observation that *his4–519 leu2–3 can1–101 SUF1* strains (His$^+$ Leu$^+$ Can^s phenotype) give rise to canavanine-resistant segregants at high frequency (Figure 2).

These segregants might arise by second-site mutations in the *can1* gene such that suppression of *can1–101* is blocked or by mutations that interfere with suppressor function. The first type of event can be distinguished from the second because second-site revertants in the *can1* gene would remain His$^+$ Leu$^+$ by

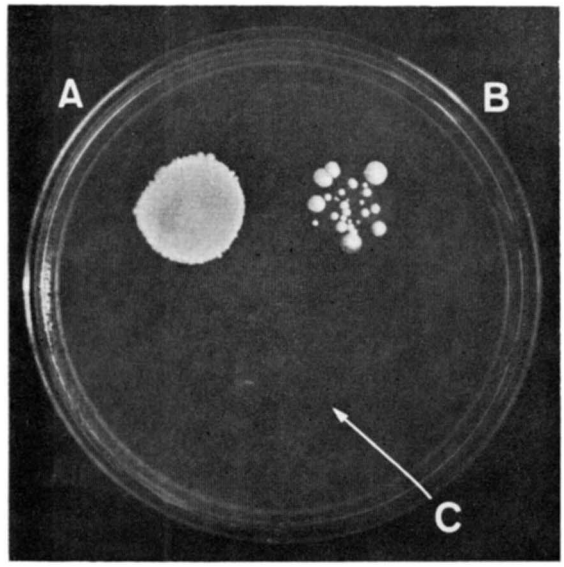

FIGURE 2.—The figure illustrates a method for estimating the extent of suppressor instability through the use of the suppressible canavanine-resistance mutation, *can1–101*. (A) A strain carrying *his4–519 leu2–3 can1–101 SUF1* was pregrown in nonselective YEPD medium. The preculture was diluted and a 100 μl drop of the suspension containing 10^4 cells was placed on a minimal + his + leu + canavanine plate. The canavanine-resistant phenotype of the strain after pregrowth in nonselective medium indicates loss of suppressor function in a high proportion of mitotic segregants. (B) The same strain was pregrown in selective (minimal) medium after which 10^4 cells of the preculture were placed on the canavanine-containing plate. Under these conditions, the strain is canavanine sensitive by virtue of suppression of *can1–101*. However, some revertants are present even after selective pregrowth, as indicated by canavanine-resistant colonies arising on the background of sensitive cells. These colonies were picked, purified and tested for their His and Leu phenotypes. All were His$^-$ Leu$^-$, indicating loss of suppressor function. (C) A strain carrying *his4–519 leu2–3 CAN1+ suf+* was pregrown in YEPD, after which 10^4 cells were placed on the canavanine-containing plate. This strain serves as a control and is stably canavanine sensitive.

virtue of a functional suppressor, whereas mutations interfering with suppressor function would result in a His$^-$ Leu$^-$ phenotype.

Several hundred independent canavanine-resistant segregants were picked, purified and tested. All of them were His$^-$ Leu$^-$, indicating that selection for canavanine resistance in *his4–519 leu2–3 can1–101 SUF1* strains provides a powerful method for the isolation of suppressor revertants.

Since absolute mutation or reversion rates are difficult to measure, we have compared the stabilities of the suppressors to the stability of the *CAN1* gene. Figure 2 shows that *his4–519 leu2–3 can1–101 SUF1* strains grown under conditions that select for the presence of *SUF1* (minimal medium) usually give about one or two canavanine-resistant colonies per 10^3 cells plated. A canavanine-sensitive strain (*his4–519 leu2–3 CAN1+ suf+*) grown in a medium supplemented with histidine and leucine gives about one canavanine-resistant colony per 10^5 cells plated. Nonselective growth of a *his4–519 leu2–3 can1–101 SUF1* strain in YEPD medium results in confluent growth on canavanine-containing medium, indicating the loss of *SUF1* function in a high proportion of mitotic segregants. In analogous experiments, it has been shown that *SUF4* and *SUF6* have a similar high degree of phenotypic instability, whereas *SUF3* and *SUF5* are relatively stable.

Instability of *SUF1*, *SUF4* and *SUF6* might be due to high rates of spontaneous reversion or to deleterious effects of the suppressors such that revertants would have a selective growth advantage. Possible deleterious effects of the suppressors have been tested by comparing the doubling time of a strain carrying *SUF1* and an isogenic Suf$^+$ revertant of the *SUF1* strain (see MATERIALS AND METHODS). The *SUF1* strain was precultured under selective conditions to ensure that the primary culture contained 100% *SUF1* cells. The culture was monitored throughout growth for the appearance of Suf$^+$ revertants. More than 99% of the cells had retained the *SUF1* phenotype (His$^+$) at the end of logarithmic growth. The time required for cell doubling of the *SUF1* strain and the Suf$^+$ revertant strain was estimated from the exponential portion of the growth curves. In both cases, the doubling time was approximately two hr, with no significant difference in growth rate between the two cultures. A control experiment was performed in which *SUF1* and Suf$^+$ cells were mixed at a ratio of 4:1 and the culture was monitored throughout exponential growth for changes in the *SUF1*/Suf$^+$ ratio. The ratio remained essentially constant over a period of ten hours of growth. From these experiments, we conclude that the high-frequency appearance of Suf$^+$ revertants is not a simple consequence of differential growth rates of *SUF1* and Suf$^+$ revertant cells. These revertants may arise as the result of some more subtle selection process or as the result of high rates of mutation at sites that affect suppressor function.

The nature of events giving rise to Suf$^+$ revertants of *SUF1* has been investigated in detail. In preliminary studies, 25 independent revertants of *SUF1* (genotype *his4–519 leu2–3 can1–101 SUF1–R*, phenotype His$^-$ Leu$^-$ Can^r) were crossed with a strain carrying *his4–519 leu2–3 can1–101 suf+* (phenotype His$^-$ Leu$^-$ Can^r). If the mutation preventing suppressor function was not linked to the

SUF1 locus, His$^+$ Leu$^+$ Can^s recombinant spores would be expected in tetrads from these crosses. In every cross, all four spores of each tetrad had a His$^-$ Leu$^-$ Can^r phenotype. Thus, none of the 25 revertants carried unlinked antisuppressor mutations.

In order to analyze large numbers of revertants, a patch test was devised to distinguish revertants carrying mutations linked to *SUF1* from those carrying unlinked mutations, and 998 independent revertants of a strain carrying *his4–519 leu2–3 can1–101 SUF1 ade2* were obtained by selection for canavanine resistance and mated with a strain carrying *his4–519 leu2–3 can1–101 suf+ lys2*. The nonsuppressible markers *ade2* and *lys2* were used to select diploids by complementation. Diploids selected in this way were sporulated. Patches of sporulated cells were then replica-plated to minimal + ade + lys medium to select for His$^+$ Leu$^+$ meiotic recombinants. Since mutations linked to *SUF1* should recombine with *SUF1* infrequently during meiosis, *SUF1* revertants carrying linked mutations should have a His$^-$ Leu$^-$ phenotype after sporulation because virtually all spores that carry *SUF1* also carry the mutation that blocks *SUF1* function. However, unlinked mutations should recombine frequently with *SUF1*, and 25% of the spores should carry a functional *SUF1* gene free of the antisuppressor mutation. Revertants of this type would have a His$^+$ Leu$^+$ phenotype after sporulation. Since all 998 revertants analyzed by this method had a His$^-$ Leu$^-$ phenotype after sporulation, we conclude that instability of *SUF1* is not due to unlinked antisuppressor mutations. Less extensive tests with *SUF4* and *SUF6* gave similar results.

Since the spot tests described above depend on a high frequency of recombination during meiosis to reveal the presence of an unlinked antisuppressor, the tests do not have the resolution to reveal antisuppressors closely linked to *SUF1* or intragenic second-site mutations. However, closely linked mutations can be revealed in patch tests designed to detect rare recombinants among meiotic spores. To examine this question, 10 independent *his4519 leu2–3 can1–101 SUF1–R* revertants were crossed with a strain carrying *his4–519 leu2–3 can1–101 suf+*. Diploids isolated by zygote manipulation were sporulated in large patches. Following sporulation, they were replica-plated to minimal medium. All of the sporulated patches of cells were phenotypically His$^-$ Leu$^-$, but after several days individual colonies were observable on the background of nongrowing cells. Five colonies from each of 10 patches corresponding to each of the 10 revertants were picked, purified and crossed with a strain carrying *his4–519 leu2–3 can1–101 SUF1*. In every case, tetrads from these crosses gave a segregation pattern of 4 His$^+$ Leu$^+$ Can^s:0 His$^-$ Leu$^-$ Can^r, suggesting that the crosses were in fact homozygous for *SUF1*. The four spores of a single tetrad from one of these crosses were tested for two other properties of *SUF1* instability and the lethal spore pattern expected when *SUF1* is crossed with *SUF4* or *SUF6*. All four spores carried an unstable suppressor that was lethal in combination with *SUF4* and *SUF6*.

If the His$^+$ Leu$^+$ phenotype of rare colonies arising after sporulation of a *SUF1–R/suf+* diploid were due to new suppressor mutations, one would not expect all 50 of the colonies analyzed to carry *SUF1*. Some should, by chance,

have carried other Group II suppressors. This argument is strengthened by the observation that *SUF1–R SUF4* and *SUF1–R SUF6* haploid strains are viable, unlike *SUF1 SUF4* or *SUF1 SUF6* haploid strains (CULBERTSON *et al.* 1977). The most likely explanation for the presence of *SUF1* in all His$^+$ Leu$^+$ colonies is that they arise by a recombination event that separates *SUF1* from a closely linked second-site mutation or by reversion of the second-site mutation, leaving a functional *SUF1* suppressor.

The 10 revertants analyzed above were crossed to each other to determine whether the second-site mutations were at the same or different sites. The crosses can be represented as *his 4–519 leu2–3 can1–101 SUF1–R$_x$* × *his4–519 leu2–3 can1–101 SUF1–R$_y$*. The diploids were analyzed by mitotic recombination and by a sporulation patch test similar to the one described above. No significant difference was observed in the number of His$^+$ Leu$^+$ colonies obtained from these diploids and homoallelic control diploids. The appearance of some His$^+$ Leu$^+$ colonies at low frequency in homoallelic controls precludes the possibility of distinguishing between reversion of the second-site mutations and low levels of recombination between very close second sites.

We conclude from these results that the instability of *SUF1* is due primarily to mutations at a second site in or near the suppressor gene or at several sites that are too close together to be distinguished by recombination in two-point crosses.

Mutations conferring an increased level of suppression: Some *his4* frameshift mutations, such as *his4–38*, are poorly suppressed by Group II suppressors to the extent that growth on medium lacking histidine is temperature sensitive. For example, strains carrying *his4–38 SUF1* are His$^+$ at 30°, but His$^-$ at 37° on minimal medium (Figure 3A, B). This temperature-sensitive phenotype is related either to the function of the suppressor or to some structural aspect of the *his4* protein produced under conditions of suppression, since *his4–38 SUF1* strains grow well at 37° on minimal plus histidine medium. *SUF4* and *SUF6* behave similarly in strains carrying *his4–38*. Furthermore, temperature-sensitive growth in these strains is an unstable phenotype, and segregants arise at high frequency that are capable of growth at 37° on minimal medium.

We have taken advantage of these observations in analyzing a set of 18 independent variants of a strain carrying *his4–38 SUF1* that were isolated by selecting for a His$^+$ phenotype at 37°. The variant strains were crossed to a temperature-sensitive *his4–38 SUF1* strain. At 30°, 4 His$^+$:0 His$^-$ segregations were observed in tetrads derived from each diploid as the result of homozygosity of *SUF1*. However, at 37°, 2 His$^+$:2His$^-$ segregations were observed (Figure 3C, D). The same *his4–38 SUF1–R* variants were then crossed to a strain carrying *his4–38 suf*$^+$. Since these crosses were heterozygous for *SUF1*, 2His$^+$:2 His$^-$ segregations were observed at 30°. At 37°, three segregation patterns were observed in an approximate 1:4:4 ratio—2:2, 0:4 and 1:3 (His$^+$:His$^-$).

Two conclusions can be drawn from these crosses. (1) The variant strains carry chromosomal mutations that segregate independently of *SUF1*. They modify the phenotype of *SUF1* by conferring growth on minimal medium at 37°, and (2) the new mutations do not result in autonomous suppression of *his4–38*

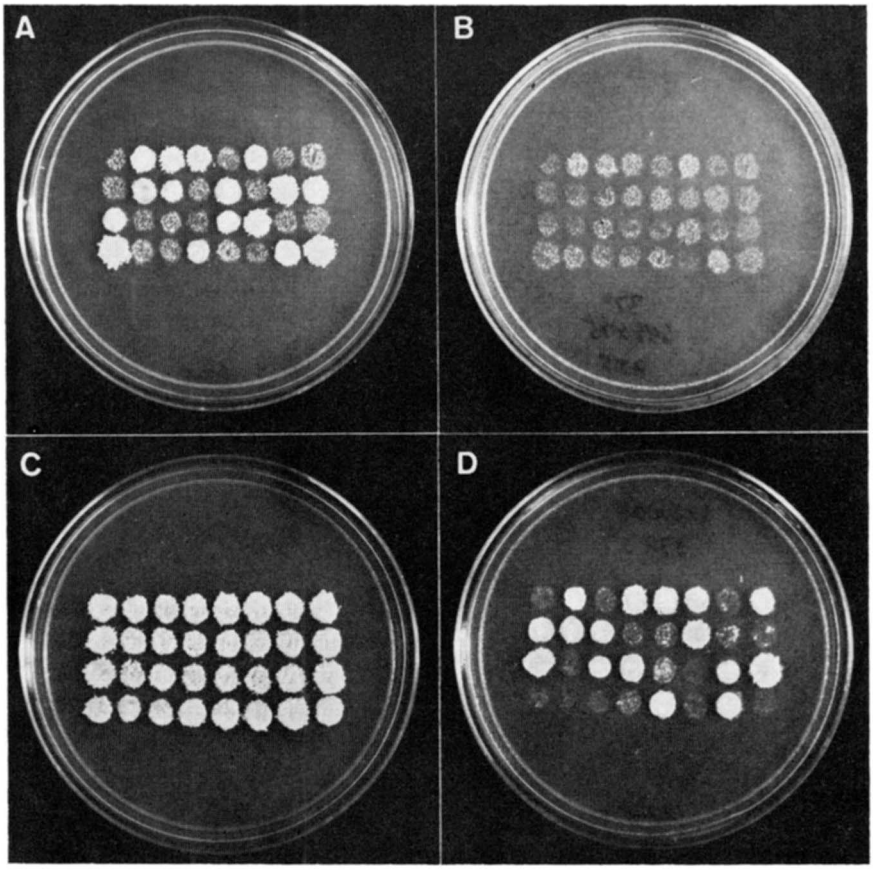

FIGURE 3.—The figure illustrates the segregation of mutations conferring enhanced suppression at an elevated temperature. (A, B) Tetrads from the cross *his4–38 suf*+ × *his4–38 SUF1* were replica-plated to minimal medium and incubated at 30° (A) and at 37° (B). The 2 His+:2 His⁻ segregations observed at 30° indicate Mendelian segregation of *SUF1*. The 0 His+:4 His⁻ segregations observed at 37° demonstrate the temperature sensitivity of the *SUF1* phenotype. All four spores of each tetrad grew at 37° on minimal + his medium (not shown). (C, D) Tetrads from the cross *his4–38 SUF1 UPF*+ × *his4–38 SUF1 upf1* were replica-plated to minimal medium and incubated at 30° (C) and at 37° (D). The 4 His+:0 His⁻ segregations observed at 30° indicate homozygosity of *SUF1*. The 2 His+:2 His⁻ segregations observed at 37° demonstrate the Mendelian inheritance of the *upf1* mutation. Thus, *upf1* confers enhanced suppression in *his4–38 SUF1* strains. Other experiments show that *upf* mutations do not suppress *his4–38* autonomously in the absence of *SUF1* (see text).

in a *suf*+ background and can be detected phenotypically only by their ability to enhance suppression in strains carrying *SUF1*. Since these mutations may act by increasing the efficiency of suppression in *SUF1* strains, this class of modifying genes has been named *upf* for "up-frameshift suppressor."

Seventeen of the 18 mutants analyzed were recessive in *his4–38 SUF1 upf/ his4–38 SUF1 UPF*+ heterozygous diploids, as indicated by a His⁻ phenotype at 37°. Complementation tests were performed in pairwise crosses with purified

diploids isolated by zygote manipulation. In the case of recessive *upf* mutants, complementation is indicated by a *His*$^-$ phenotype at 37°, whereas failure to complement is indicated by a His$^+$ phenotype at 37°. These tests revealed the existence of two groups of complementing mutants. Group I consisted of 14 representatives and Group II consists of three. One representative from each group was analyzed in detail (Table 3). Crosses 1 and 3 of Table 3 demonstrate Mendelian segregation of both *upf* mutations. Crosses 2 and 4 show that neither of the two mutations is linked to *SUF1*. When strains carrying each of the *upf* mutations were crossed to each other (Cross 5), recombinants were observed in the proportions expected for two unlinked genes. Thus, these crosses define two genes: *upf1* (complementation Group I) and *upf2* (complementation Group II).

One of the 18 mutants analyzed was dominant in a heterozygous diploid. To determine whether this mutant carried an allele of *upf1*, *upf2* or a third *upf* gene, the mutant was crossed with *his4–38 SUF1* strains carrying *upf1* or *upf2*. In tetrads from the *upf1* cross, 4 his$^+$:0 His$^-$ segregations were observed at 37°. In the *upf2* cross, 4:0, 2:2 and 3:1 (His$^+$:His$^-$) segregations were observed at 37° in the ratio expected for two unlinked genes. Thus, the dominant *upf* mutant behaves as an allele of the *upf1* locus.

Considering the numerous similarities between *SUF1*, *SUF4* and *SUF6* and the likelihood that these suppressors act through the same tRNA, *upf1* and *upf2* mutations isolated in *his4–38 SUF1* strains were tested for their ability to enhance suppression in *SUF4* and *SUF6* strains. The properties of the suppressors themselves render it difficult to demonstrate enhanced suppression, since pairwise crosses involving these suppressors result in a lethal segregation pattern in which all double-mutant spores carrying two suppressors are inviable (CULBERTSON *et al.* 1977). To obviate this difficulty, *his4–38 suf*$^+$ *upf1* (or *upf2*) strains were constructed and then crossed to *his4–38 SUF4* and *his4–38 SUF6* strains. The appropriate strain construction was accomplished by crossing *his4–38 SUF1* strains carrying recessive *upf* mutations to a strain carrying *his4–38 suf*$^+$ *UPF*$^+$. *his4–38 suf*$^+$ strains carrying the *upf* mutations were identified among spore clones from the crosses. Although the *upf* phenotype cannot be assayed in a *suf*$^+$ background, spores of the appropriate genotype could be identified in tetrads that segregated 2:2 (His$^+$:His$^-$) at 30° and 0:4 at 37°. Spores from these tetrads that had a His$^-$ phenotype at both temperatures were assumed to have the genotype *his4–38 suf*$^+$ *upf1* (or *upf2*). This was confirmed by tetrad analysis of crosses between these strains and a strain carrying *his4–38 SUF1 UPF*$^+$. *his4–38 suf*$^+$ *upf1* and *his4–38 suf*$^+$ *upf2* strains were then crossed to temperature-sensitive *his4–38 UPF*$^+$ strains carrying *SUF4* or *SUF6*. Crosses 6, 7, 8 and 9 of Table 3 show that *upf1* and *upf2* confer growth at 37° in *SUF4* and *SUF6* strains. Furthermore, these crosses show that *upf1* and *upf2* recombine with *SUF4* and *SUF6* in the ratios expected for unlinked genes. Thus, *upf1* and *upf2* are not alternative alleles of *SUF4* or *SUF6*.

Similar experiments were performed to determine whether *upf1* and *upf2* enhance suppression in strains carrying *SUF3* or *SUF5*. In preliminary experi-

TABLE 3

Interaction of upf (*"up-frameshift suppressor"*) *mutations with* SUF1, SUF4 *and* SUF6

Cross	Parental genotypes	Segregation on minimal medium* 30° His+:His-	37° His+:His-	Conclusions
1	*his4-38 SUF1 upf1* × *his4-38 SUF1 UPF+*	4:0(12)	2:2(12)	Mendelian inheritance of *upf1*
2	*his4-38 SUF1 upf1* × *his4-38 suf+ UPF+*	2:2(24)	2:2(5), 0:4(4), 1:3(15)	nonlinkage of *upf1* and *SUF1*
3	*his4-38 SUF1 upf2* × *his4-38 SUF1 UPF+*	4:0(12)	2:2(12)	Mendelian inheritance of *upf2*
4	*his4-38 SUF1 upf2* × *his4-38 suf+ UPF+*	2:2(22)	2:2(4), 0:4(5), 1:3(13)	nonlinkage of *upf2* and *SUF1*
5	*his4-38 SUF1 upf1* × *his4-38 SUF1 upf2*	4:0(23)	4:0(4), 2:2(2), 3:1(15)	nonlinkage of *upf1* and *upf2*
6	*his4-38 suf+ upf1* × *his4-38 SUF4 UPF+*	2:2(18)	0:4(2), 2:2(2), 1:3(14)	nonlinkage of *upf1* and *SUF4*
7	*his4-38 suf+ upf1* × *his4-38 SUF6 UPF+*	2:2(20)	0:4(4), 2:2(2), 1:3(14)	nonlinkage of *upf1* and *SUF6*
8	*his4-38 suf+ upf2* × *his4-38 SUF4 UPF+*	2:2(24)	0:4(4), 2:2(4), 1:3(16)	nonlinkage of *upf2* and *SUF4*
9	*his4-38 suf+ upf2* × *his4-38 SUF6 UPF+*	2:2(21)	0:4(3), 2:2(3), 1:3(14)	nonlinkage of *upf2* and *SUF6*

* Numbers in parentheses indicate numbers of tetrads analyzed.

TABLE 4

Interaction of upf *mutations with* SUF3 *and* SUF5

Cross	Segregation on minimal medium*		
	23° His+:His-	30° His+:His-	37° His+:His-
his4–38 suf+ upf1 × *his4–38 SUF3 UPF+*	2:2(22)	2:2(22)†	0:4(22)
his4–38 suf+ upf2 × *his4–38 SUF3 UPF+*	2:2(23)	2:2(23)	0:4(4), 2:2(6), 1:3(13)
his4–38 suf+ upf1 × *his4–38 SUF5 UPF+*	2:2(22)	0:4(4), 2:2(2), 1:3(16)	0:4(4), 2:2(2), 1:3(16)
his4–38 suf+ upf2 × *his4–38 SUF5 UPF+*	2:2(20)	0:4(20)	0:4(20)

* Numbers in parentheses indicate numbers of tetrads analyzed.

† Although these tetrads segregated 2 His+ : 2 His-, some of the spores were more strongly His+ than others. Thus, *upf1* may enhance suppression in strains carrying *SUF3* to some extent, but the enhancement is not sufficient to confer growth at 37°.

The results show that *upf2* enhances suppression in strains carrying *SUF3*, whereas *upf1* enhances suppression in strains carrying *SUF5*.

ments, it was found that *his4–38 SUF3* strains are phenotypically His^+ at 30° and His^- at 37°. *SUF5*, the weaker of the two suppressors, confers a His^+ phenotype at 23° and a His^- phenotype at 30° and 37° in *his4–38* strains. Table 4 shows the results of crosses between strains carrying *upf* mutations and strains carrying *SUF3* or *SUF5*.

Enhancement of suppression in the *SUF3* crosses was assayed by testing for the ability of spore clones from tetrads to grow at 37° on minimal medium. 2 His^+:2 His^- segregations were observed at 23° and 30°, since *SUF3* was heterozygous in these crosses. The *upf1* × *SUF3* cross failed to give His^+ segregants at 37°. Thus, we tentatively conclude that *upf1* fails to enhance suppression in strains carrying *SUF3*. However, in the tetrads that segregated 2:2 at the lower temperatures, both weak and strong His^+ phenotypes were observed. This may be the result of weak enhancement of suppression by *upf1*. The *upf2* × *SUF3* cross resulted in 0:4, 2:2 and 1:3 (His^+:His^-) segregations at 37°, suggesting that *upf2* enhances suppression in strains carrying *SUF3*; *upf2* and *SUF3* are not linked.

Enhancement of suppression in the *SUF5* crosses was assayed by testing for the ability of spore clones from tetrads to grow at 30° on minimal medium. 2 His^+:2 His^- segregations were observed at 23°, since *SUF5* was heterozygous in these crosses. The *upf1* × *SUF5* cross resulted in 0:4, 2:2 and 1:3 (His^+:His^-) segregations at 30°, suggesting that *upf1* enhances suppression in strains carrying *SUF5*; *upf1* and *SUF5* are not linked. In the *upf2* × *SUF5* cross, 0:4 segregations were observed at 30°. Thus, *upf2* fails to enhance suppression in strains carrying *SUF5*.

Since the assay for enhancement of suppression depends on recombination between the *upf* mutation and the suppressor, the above results might be erroneous in cases where no enhancement was observed, for example, if a *upf* mutation were tightly linked to the suppressor. Linkage of *upf2* to the *SUF5* locus was tested by determining whether *upf2* was linked to the *ade2* locus on chromosome *XV*. *SUF5* was shown above to map 12 cM from *ade2* (Table 2; Figure 1). Analysis of tetrads from the cross *his4–38 SUF1 upf2 ADE2*$^+$ × *his4–38 SUF1 UPF*$^+$ *ade2* revealed that *upf2* and *ade2* are unlinked. Thus, *upf2* is not an alternative allele of the *SUF5* locus and fails to enhance suppression in strains carrying *SUF5*. Since *SUF3* has not yet been mapped, it was not possible to perform a similar linkage test between *upf1* and *SUF3*.

In summary, the results indicate that *upf1* enhances suppression in strains carrying *SUF1*, *SUF4*, *SUF5* or *SUF6*, and *upf2* enhances suppression in strains carrying *SUF1*, *SUF3*, *SUF4* or *SUF6*.

Increased efficiency of suppression due to the cytoplasmically inherited [PSI] element can be detected by the same methods that revealed the existence of *upf1* and *upf2* (CULBERTSON *et al.* 1977). *his4–38 SUF1* [PSI^-] strains that are temperature sensitive for growth on minimal medium lose their temperature-sensitive phenotype when [PSI] is introduced in a cross. Unlike *upf* mutations, enhancement of suppression in [PSI^+] strains is restricted to the suppressors

SUF1, *SUF4* and *SUF6*. Another distinction between [PSI] and *upf* mutations is apparent in crosses homozygous for *his4–38* and *SUF1*. Heterozygosity for a *upf* mutation in such a cross results in a 2 His^+:2 His^- segregation at 37°. "Heterozygosity" for the [PSI] element results in a non-Mendelian 4 His^+:0 His^- segregation at 37°. The possibility of a relationship or interaction between *upf* mutations and the [PSI] element is being investigated.

DISCUSSION

Analysis of frameshift revertants: Some of the genetic properties of Group II frameshift suppressors in *Saccharomyces cerevisiae* are described in this communication. Suppressor mutations mapping at five different loci were obtained by coreversion of the ICR-170-induced mutations, *his4–519* and *leu2–3*. The spectrum of coreversion events was dependent on the method by which they were induced. ICR-induced corevertants carried suppressors that mapped at the *SUF3* and *SUF5* loci. Spontaneous and UV-induced corevertants were rare. Four of the five that were analyzed carried alleles of *SUF1*, *SUF4* and *SUF6*.

The spectrum of mutations induced by ICR-170 observed in this study resembles that observed in another study on revertants of the Group III, ICR-induced mutation *his4–713* (CUMMINS *et al.* 1980). All of the ICR-induced revertants of *his4–713* map at the *SUF2* locus near the centromere of chromosome *III*. Nonetheless, five additional suppressor loci were identified among spontaneous and UV-induced revertants. Thus, only three (*SUF2*, *SUF3* and *SUF5*) of the 11 known suppressor loci are frequent targets for mutagenesis by ICR-170.

These results probably reflect the sequence specificity of ICR-170 for monotonous runs of G/C base pairs (AMES and WHITFIELD 1966; YOURNO and HEATH 1969; YOURNO 1971). It has been shown that three consecutive G/C base pairs is sufficient to serve as a target site in ICR mutagenesis (RIDDLE and CARBON 1973). Perhaps some of the suppressor loci in yeast that are not highly susceptible to induction by ICR-170 fail to meet the minimum consecutive G/C base pair requirement. For example, +1 G/C suppressor mutations in glycyl-tRNA genes coding for a tRNA with CCC at the anticodon may be induced by ICR-170, producing a CCCC anticodon. Other glycyl-tRNA genes coding for a tRNA with CCU at the anticodon may not be susceptible to induction by ICR-170. Nonetheless, base insertions resulting in a suppressor phenotype might be obtained among spontaneous or UV-induced revertants of frameshift mutations because the mechanisms by which mutations are generated using these methods are different from that of ICR-170.

In the course of these studies, one of the Group II suppressors, *SUF5*, was mapped on chromosome *XV* between *ade2* and *ade9*. In other studies, five of the six Group III suppressors were mapped (CULBERTSON *et al.* 1977; CUMMINS *et al.* 1980). Thus far, the map locations of the suppressors indicate that external revertants of ICR-induced mutations carry new suppressors not identified in previous studies on nonsense or omnipotent suppressors.

Analysis of mutations affecting suppressor efficiency: The stability of Group II suppressors was examined in detail by developing a system in which revertants of the suppressors could be obtained by positive selection. This was accomplished by isolating an ICR-induced mutation conferring resistance to L-canavanine. The mutation, designated *can1–101*, is suppressed by *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*. Revertants of the suppressors were obtained by selecting for canavanine resistance in strains carrying a suppressed *can1–101* mutation.

Using the canavanine selection system, it was found that *SUF3* and *SUF5* are phenotypically stable, whereas *SUF1*, *SUF4* and *SUF6* are highly unstable. High-frequency reversion resulting in loss of suppressor function in strains carrying *SUF1*, *SUF4* or *SUF6* may be due to mutations affecting transcription, processing or base modification of the suppressing tRNA. An extension analysis of revertants that harbor nonfunctional suppressors revealed that reversion is due to mutations at sites in or adjacent to the suppressor genes themselves. Although revertants of *SUF1* were examined in much greater detail than those of *SUF4* and *SUF6*, the results for all three suppressors suggest the presence of a single site tightly linked to each suppressor locus that can be altered by mutation in such a way as to destroy suppressor function. Mutational alterations at these sites could lower the efficiency of transcriptional promotors, disrupt enzyme-substrate recognition during processing or post-transcriptional base modification, or disrupt tRNA conformation and result in loss of suppressor function.

The instability of *SUF1*, *SUF4* and *SUF6* might be due to excessively high mutation rates at intragenic sites that, when mutated, destroy suppressor function. Alternatively, the suppressors might have deleterious effects on the overall growth rate of cells harboring a suppressor such that Suf$^+$ revertants are represented in cultures grown under nonselective conditions at high frequency due to their comparatively rapid doubling time. In order to test these possibilities, we have compared the doubling times of a *SUF1* strain and an isogenic Suf$^+$ revertant of the *SUF1* strain. The doubling times of the two strains were roughly equivalent. A reconstitution experiment in which *SUF1* and Suf$^+$ cells were mixed and grown together in the same culture confirmed this result. Thus, it appears unlikely that high-frequency reversion of *SUF1* is due to a simple process of selection based on a growth advantage of Suf$^+$ revertants. Considering the difficulty in providing quantitative data on the rate of reversion, we wish to exercise caution in stating that elevated mutation rates may be the cause of suppressor instability. Further growth tests using a variety of growth conditions will be required in order to rule out the selection model.

The nature of the second-site mutations that abolish suppressor function is unknown. Since genetic instability is characteristic of tandemly duplicated genes, both in yeast and bacteria (HINNEN, HICKS and FINK 1978; ANDERSON and ROTH 1977), suppressor instability might be due to unequal crossing over in tandemly duplicated regions. According to this model, unequal crossing over between adjacent homologous segments of DNA would result in deletion of the suppressor mutation. Our data do not seem to support this model, since the sup-

pressor mutations can be recovered from revertants by recombination and are therefore not deleted. However, another more complicated model is formally possible. A tandemly duplicated region might contain repeated copies of the suppressor mutation itself. Unequal crossing over could delete some of the additional copies, leaving a copy number of suppressor mutations that is insufficient to confer a suppressor phenotype due to reduced efficiency of suppression. Reamplification of the duplicated region might account for the ability to recover the suppressor phenotype in diploids. We consider this possibility remote and are led at this time to the simplest view that reversion is due to mutations affecting the synthesis or function of the suppressing tRNA rather than to deletions of tandemly duplicated DNA.

Frameshift suppression is enhanced in strains carrying mutations in two genes, designated *upf1* and *upf2*. These "up-frameshift suppressor" mutations have been characterized by (1) their inability to act as autonomous suppressors of frameshift mutations in the absence of a frameshift suppressor, (2) their ability to enhance suppression in strains carrying *SUF1*, *SUF4* or *SUF6*, (3) the ability of *upf1* to enhance suppression in strains carrying *SUF5*, but not *SUF3*; and the ability of *upf2* to enhance suppression in strains carrying *SUF3*, but not *SUF5*, and (4) the lack of genetic linkage of *upf1* and *upf2* mutations to each other or to any of the Group II suppressor loci (with the possible exception of *upf1* and *SUF3*, which could not be tested). Mutations mapping at the *upf1* and *upf2* loci occur at high frequency in strains carrying *SUF1*, *SUF4* or *SUF6*.

The interaction of *upf* mutations with *SUF1*, *SUF4* and *SUF6* serves to reemphasize the relationship between these suppressors. Evidence is provided in Table 1 that *SUF1*, *SUF4* and *SUF6* comprise a distinct subset of suppressors, since they differ from *SUF3* and *SUF5* in regard to their induction with mutagens. Considering the other properties common to *SUF1*, *SUF4* and *SUF6*, including lethality in haploid pairwise combinations, enhanced suppression in [PSI$^+$] strains, reduced isoacceptor activity of tRNA^{GLY3} and genetic instability, these suppressors may represent redundant genes coding for the same tRNA.

Several different molecular interpretations of the interaction between *upf* mutations and frameshift suppressors can be proposed on the basis of an analysis of the selection system used to obtain these mutants. *upf* mutations were obtained by selection for growth at 37° in *his4–38 SUF1* strains that are temperature sensitive for growth on minimal medium. Although the mechanism responsible for temperature sensitivity in these strains is not known, two alternative models account for the observations. The first model assumes that the suppressed *his4* protein in a *his4–38 SUF1* strain contains an altered amino acid sequence that renders the protein thermolabile or susceptible to degradation by proteases. The altered amino acid sequence might be generated by suppression and restoration of the correct reading frame at a site downstream from the site of the *his4–38* mutation. The second model assumes that the suppressed *his4* protein in a *his4–38 SUF1* strain is temperature independent for its activity by virtue of a normal amino acid sequence, but the altered transfer RNA responsible for sup-

pression of *his4–38* is temperature sensitive for its function in protein synthesis.

Assuming that the first model is correct, *upf* mutations that restore histidine independent growth at the elevated temperature might be regulatory mutations specifically involved in histidine biosynthesis or protease mutations that stabilize the *his4* protein under conditions of suppression. Assuming that the second model is correct, *upf* mutations might interact with the suppressing tRNA by affecting tRNA transcriptional, processing or base modification reactions, or the efficiency of aminoacylation of the tRNA.

These two models for the *upf*-frameshift suppressor interaction might be distinguished directly by determining the nature of the *his4–38* mutation. Preliminary data from sequence analysis of a cloned DNA fragment carrying the *HIS4* region indicates the presence of an in-frame GGC glycine codon in the region expected to contain the *his4–38* mutation (FARABOUGH, DONAHUE and FINK, unpublished). Cloning and sequencing of the corresponding DNA fragment from the *his4–38* mutant is in progress.

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