

RECESSIVE LETHAL AMBER SUPPRESSORS IN YEAST

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Manuscript received June 10, 1974

Revised copy received December 4, 1974

ABSTRACT

Recessive lethal amber suppressor mutations have been isolated in a diploid strain of *Saccharomyces cerevisiae*. Diploids carrying these suppressors upon sporulation yield asci with only two live spores, both lacking the suppressor. At least two classes of recessive lethal suppressors exist. Aneuploid strains carrying one wild type and one suppressor locus have been isolated and used in mapping studies; one suppressor maps on chromosome III, the other does not.

SUPPRESSOR mutations capable of reversing the phenotypes of ochre or amber alleles in the yeast *Saccharomyces cerevisiae* were first described by HAWTHORNE and MORTIMER (1963). These suppressor mutations, isolated in haploid strains of yeast, are generally assumed to be analogous to the suppressor mutations in *E. coli* which have been shown to be altered tRNAs (MANNEY 1964; GILMORE 1967; HAWTHORNE and MORTIMER 1968; GILMORE, STEWART and SHERMAN 1971; STEWART and SHERMAN 1972; STEWART *et al.* 1972). The best studied group of yeast suppressors, the class I set 1 suppressors in GILMORE's (1967) classification system, recognizes the ochre codon and inserts tyrosine into protein (GILMORE, STEWART and SHERMAN 1971). In an attempt to generate new classes of suppressors which insert different amino acids into protein, suppressors were isolated in diploid yeast strains.

This paper describes the isolation of a new class of suppressors which cannot be isolated or maintained in a haploid strain. These suppressors are thus analogous to the recessive lethal suppressors isolated in merodiploids of *E. coli* (SOLL and BERG 1969) and *S. typhimurium* (MILLER and ROTH 1971).

MATERIALS AND METHODS

Yeast strains and nomenclature: The heterothallic strains of *Saccharomyces cerevisiae* used in this study were derived from strains provided by DR. F. SHERMAN and DR. G. FINK. The symbols and nomenclature are those proposed at the IVth and Vth International Yeast Genetics Conference (VON BORSTEL 1969). *SUP* is the dominant suppressor mutation and *sup*⁺ refers to the wild-type suppressor-less condition. Each strain number is preceded by a letter which denotes its ploidy: DBH for haploids, DBD for diploids, and DBA for aneuploids.

The parental diploid DBD195 was constructed by mating the two haploid strains, DBH249, a *his1 leu2-1 trp1-1 tyr7-1 CAN*^s *sup*⁺ and DBH250, α *ade1 leu2-1 trp1-1 tyr7-1 can^r sup⁺*.

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Leu2-1 is an ochre allele, *trp1-1* and *tyr7-1* are amber mutations while the *ade1* and *his1* markers are non-suppressible. Resistance to canavanine is recessive and was not reversed by the introduction of a suppressor. All strains are *psi⁻* (Cox 1965).

Media: The culture media used and their purposes are described below.

Rich medium (YEP-glucose): 1% Difco Yeast Extract, 2% Difco Bacto Peptone, 2% glucose, 2% Difco Bacto-agar or Fisher BBL agar (omitted in liquid media). This is a complete medium and was used for propagation of most cultures, except when maintenance of aneuploidy was desired.

Minimal medium (SD): 0.67% Difco Yeast Nitrogen base (without amino acids), 2% glucose, 3% Difco Bacto-agar or Fisher BBL agar (omitted in liquid media). When required, the following concentrations of supplements were added: adenine sulfate, 20 mg/l; L-histidine-HCl, 20 mg/l; L-leucine, 30 mg/l; L-lysine-HCl, 30 mg/l; L-methionine, 20 mg/l; L-phenylalanine, 50 mg/l; L-tryptophan, 20 mg/l; L-tyrosine, 30 mg/l; L-canavanine sulfate, 60 mg/l. Supplemented minimal medium was used for the culture of aneuploids and as a control in scoring nutritional phenotypes.

Incubation conditions: All incubations were at 30°. For liquid cultures, flasks or tubes were aerated on rotary or reciprocating shakers.

Genetic methods:

Mating: Stationary phase cultures of haploid strains of opposite mating types were mixed together in a spot on YEP-glucose and replicated to selective plates, or spotted directly on selective minimal plates. In order to isolate progeny from rare matings between a/α aneuploids and a or α haploids, 0.4 ml of the mating mixture was spread on appropriately supplemented selective minimal media. Diploids were purified by subcloning on selective media.

Sporulation: Cultures were grown for 48 hours into stationary phase, washed once in water, resuspended in 0.3% potassium acetate and then diluted 1:10 into the same medium. They were then incubated with aeration at 30° until asci appeared (usually two days). The sporulated cultures could be stored in water at 4° indefinitely.

Tetrad analysis: After sporulation, the asci were dissected by the procedure of JOHNSTON and MORTIMER (1959) using Glusulase (Endo Laboratories) to digest the ascus walls. The genotype of each spore was determined by replica-plating to appropriately supplemented minimal media. Complementation tests were used to determine the mating type of each spore and to discriminate markers of like phenotype.

Before the induction of suppressor mutations, the parent diploid DBD195 was subjected to tetrad analysis. Spore viability was greater than 90%. The heterozygous markers segregated 2+ : 2- and the homozygous markers segregated 0+ : 4-, as expected. After induction of a recessive lethal suppressor mutation, the diploids yielded many asci with fewer than four live spores. The decrease in spore viability was attributed to segregation of the lethal mutation. (Only those asci which had the four spores successfully dissected were used in genetic analysis.) In any given cross between a *SUP/sup⁺* aneuploid (disomic for the chromosome carrying the *SUP* marker) and a tester haploid, the average frequency of tetrads in which all four spores germinated was 0.1. Only tetrads with three and four viable spores per tetrad were counted in genetic analyses.

Random spore analysis: Diploids were analyzed by the random spore method of GILMORE (1967) with slight modifications. After sporulation, approximately 10^6 cells were treated with Glusulase for 1 hour and then diluted 1:50 in water. The suspension was sonicated using a Heat Systems Company-Ultrasonics, Inc. sonifier cell disrupter model W185 set at an output of 50-70 watts, using a standard microtip. Sonication time was 2 minutes (15-second intervals with 30-second cooling periods), and the spores were diluted and plated on appropriately supplemented minimal medium containing canavanine. Since resistance to canavanine is recessive, only haploids arising from *can^r* spores and rare *can^r/can^r* homozygotes could form colonies.

Mitotic segregational analysis: This test was performed to quantitate the instability of the *SUP*-bearing chromosome in putative aneuploid strains. Single colonies of the strains to be tested were grown up in selective medium, diluted 100-fold into YEP broth and grown until stationary phase was reached. The cells were titered on YEP plates. 200 to 300 colonies were replica-plated and scored for loss of the suppressor and for mating type. The segregants which had lost the suppressor usually formed much larger colonies than their *SUP/sup⁺* parents.

Aneuploid mapping: Trisomic analysis was used to determine which chromosomes were duplicated in the aneuploid *SUP* strains (SHAFFER *et al.* 1971; MORTIMER and HAWTHORNE 1973). When haploid strains carrying mutations in mapped genes were mated to these aneuploids, the resulting trisomic progeny were expected to yield asci with 4+:0-, 3+:1-, and 2+:2- ratios for any gene located on the same chromosome as was duplicated in the aneuploid. In order to see the aberrant 4+:0- and 3+:1- tetrad ratios, the trisomic configuration for the mapped gene was +/+/-; both + alleles deriving from the aneuploid and the - allele from the tester haploid for the given tester mutation. The tester strain also carried *trp1-1*, a suppressible amber allele which permitted scoring for the presence of the suppressor mutation.

RESULTS

Isolation of amber suppressor mutations in diploids: Single clones of the diploid DBD195, $\frac{a \text{ his1} + \text{leu2-1 trp1-1 tyr7-1 CAN}^s \text{ sup}^+}{\alpha + \text{ade1 leu2-1 trp1-1 tyr7-1 can}^r \text{ sup}^+}$, were inoculated into rich medium. After growth to stationary phase, about 4×10^7 cells from each independent culture were spread on plates lacking tryptophan, tyrosine, and phenylalanine (an additional requirement of *tyr7* mutants) to select simultaneous reversion of the amber (*trp* and *tyr*) markers. When ethyl methane sulfonate (EMS) was used to induce mutations, one drop was placed in the center of each plate.

Colonies appearing were assumed to bear suppressors since the frequency was far greater than that expected for simultaneous reversion to wild-type of both *trp1-1* and *tyr7-1*. These strains do, in fact, carry suppressors since tryptophan and tyrosine-requiring cells segregate from suppressor-bearing aneuploid strains derived from the diploids. These aneuploid strains will be described more fully in a later section.

Determination of recessive lethality by random spore screening: A random spore analysis was carried out to determine whether the suppressors were lethal or non-lethal in haploid progeny. The method takes advantage of the heterozygosity of the diploid for a recessive *can*^r marker: *CAN*^s/*can*^r diploids which have failed to sporulate and all *CAN*^s spores fail to form colonies in the presence of canavanine. Only *can*^r spores and rare *can*^r/*can*^r diploids can grow on the canavanine plates.

The diploid *SUP* colonies were purified, cultured and sporulated. Mixtures of spores were sonicated and plated on two kinds of canavanine-containing minimal media: selective minimal medium containing no tryptophan, tyrosine, or phenylalanine (selects *SUP can*^r) and supplemented medium (permits the growth of both *SUP can*^r and *sup*⁺ *can*^r). A comparison was made between the number of colonies appearing on the two types of plates. If a *non-lethal suppressor* is segregating, *SUP can*^r spores will appear on the minimal plates lacking aromatic amino acids and both *SUP can*^r and *sup*⁺ *can*^r spores will grow on the fully supplemented plates. If the diploid contains a *recessive lethal suppressor*, only *sup*⁺ haploids will be viable and such cells will grow only on the fully supplemented medium. Minimal plates lacking aromatic amino acids will show no spore growth if a recessive lethal suppressor is segregating.

Frequency of recessive lethal suppressors in diploid strains: From each independent *sup*⁺ parent diploid culture, 2-4 single *SUP* colonies were examined for

recessive lethality. Among 33 cultures containing suppressor mutations which arose spontaneously, 90% of all the suppressors were recessive lethal. From 47 suppressor-bearing cultures which had been treated with EMS, 67% of the colonies tested had recessive lethal suppressors. A similar frequency of recessive lethality among suppressors was found in diploids carrying another pair of amber mutations, *his4-580* and *met8-1*. In diploid strains, it seems that the recessive lethal class of suppressors is far more frequent under our conditions, than the non-lethal class. SOLL and BERG (1969) reported a similar high frequency of recessive lethal suppressors in merodiploids of *E. coli*.

Asci from diploids which random spore analysis indicated carried a recessive lethal suppressor were dissected. Each ascus bore two live and two dead spores. Rarely, an ascus with three viable spores occurred; without exception, all three spores lacked the suppressor mutation. The surviving spores were all *leu2-1 trp1-1 tyr1-7 sup+*. The *ade1*, *his1*, and *can* alleles segregated independently. Data for a representative sample of SUP diploid isolates is shown in Table 1.

Isolation of aneuploid strains carrying recessive lethal suppressors: Haploid spores which have inherited a recessive lethal suppressor from a heterozygous diploid do not grow. However, a spore which contains such a suppressor as well as a *sup+* copy of the suppressor locus should survive. Such rare spores can arise from meiotic nondisjunction (aneuploids), translocation, or genetic duplication, and might be detected in the random spore analysis of a diploid carrying a recessive lethal suppressor. The only colonies which can grow on the selective minimal

TABLE 1

Segregation of markers from diploids carrying recessive lethal suppressors

SUP diploid isolate	Number asci*	Number of spores: live:dead	<i>α:a</i>	<i>ade⁺:ade1</i>	<i>his⁺:his1</i>	<i>leu⁺:leu2-1</i>	<i>sup⁺:SUP†</i>
339	6	12:12	7:5	7:5	5:7	0:12	12:0
340	9	17:19	6:11	11:6	10:7	0:17	17:0
341	10	14:26	8:6	6:8	7:7	0:14	14:0
342	11	23:21	11:12	11:12	10:13	0:23	23:0
343	10	20:20	9:11	9:11	9:11	0:20	20:0

The genotype of the diploid is $\frac{a\ his1}{\alpha} + \frac{leu2-1\ trp1-1\ tyr7-1\ CAN^s\ sup^+}{\alpha + ade1\ leu2-1\ trp1-1\ tyr7-1\ can^r\ SUP}$

Each ascus yielded two live and two dead spores, with one exception, in strain DBD342. Here, one ascus gave 3 viable spores, all of which lacked the suppressor mutation. No spores carrying the suppressor mutation were ever detected in tetrad analysis. The actual tetrad viability rates were:

Strain	Total*	Live:Dead				
		4:0	3:1	2:2	1:3	0:4
339	6	0	0	6	0	0
340	9	0	0	8	1	0
341	10	0	0	6	2	2
342	11	0	1	10	0	0
343	10	0	0	10	0	0

* Some asci gave only one viable spore, a few gave no viable spores.

† The suppressor mutation is scored by counting *Trp⁺ Tyr⁺* spores.

canavanine plates are diploids which have converted or mutated the *CAN^s* locus to become *can^r/can^r*, and "haploids" which have become heterozygous for the recessive lethal suppressor locus.

The number of *can^r SUP* spores (possible aneuploids) on the minimal plate was usually less than 0.1% of the number of colonies appearing on the supplemented plate. Such colonies were purified by subcloning, cultured, and tested for the ability to sporulate; those which sporulated were presumed to be diploids and were discarded. The remaining strains were subjected to further genetic analysis.

Among the original recessive lethal suppressor-bearing diploids, two groups were seen. One type of diploid generated, when sporulated, "aneuploid" progeny which either displayed a single mating type (**a** or α) or were non-mating. The other type generated only aneuploids which displayed a single mating type (**a** or α).

A recessive lethal amber suppressor on chromosome III: Strain DBA309 was isolated by the random spore technique from *SUP* diploid DBD 339, as a single colony growing on a minimal plate containing canavanine. It did not sporulate and was further characterized.

Spot-mating tests indicated that strain DBA309 would not mate with *sup⁺ trp1-1 tyr7-1* tester strains of either mating type to produce tryptophan and tyrosine-independent (i.e., *SUP/sup⁺*) diploids. When strain DBA309 was grown to stationary phase in rich medium, where the *SUP* allele is not required, *sup⁺* cells (*Trp Tyr⁻*) appeared at a frequency of 0.4% and these *sup⁺* segregants all displayed the α mating type. No colonies were seen which could mate and were still *SUP*, or which became *sup⁺* and failed to mate as α . This suggests that strain DBA309 is aneuploid for both the mating-type locus (chromosome III) and the suppressor locus. The observed cosegregation further suggests that strain DBA309 is aneuploid for all or part of chromosome III and that the suppressor (called *SUP-RL1*) is included in the duplicated region. Note, however, that no linkage of *SUP* to mating type was detected in the tetrad analysis (Table 1). To confirm the aneuploidy of chromosome III, strain DBA309 was forced to mate with strain DBH49, **a** *his4-280 trp1-1 CAN^s sup⁺*, with selection to maintain the suppressor mutation. The plate mating yielded recombinant progeny at a frequency of 10^{-5} – 10^{-6} . If the resulting progeny are trisomic for one or more linkage groups, then heterozygous markers on the affected chromosome should frequently segregate aberrantly during meiosis. Tetrad analysis of one such strain is presented in Table 2; the result demonstrated that *his4*, mating type and the *SUP* locus all segregate aberrantly.

His4 is located on chromosome III, and the fact that 6 out of the 11 tetrads which had more than two viable spores gave 4⁺:0⁻ and 3⁺:1⁻ ratios implies that the strain is a +/+/- trisome for this locus. Mating type and *leu2-1* are also located on chromosome III. The presence of non-mating spores indicated that more than 2 copies of the mating-type locus were present. The trisomic configuration $\alpha/\alpha/\mathbf{a}$ is suggested by the appearance of many more spores which mated as α (either α or α/α) than those mating as **a**. This further suggests that the putative trisomic strain arose from the mating of DBH49 with a variant of the aneuploid

TABLE 2

Aneuploidy of chromosome III in strains DBA309 and DBA338

	Fraction of tetrads segregating aberrantly (4:0 + 3:1)	
	DBA309 × DBH49	DBA338 × DBH49
Asci with more than 2 live spores*	11/22	22/54
<i>his</i> ⁺ : <i>his4-280</i>	6/11	0/22
<i>leu</i> ⁺ : <i>leu2-1</i>	0/11	0/22
Asci with non-mating spores†	6/11	0/22

The spore progeny of a rare mating between DBA309 or DBA338 and DBH49 were dissected and characterized. *Trp*⁺ spores indicated the presence of *SUP-RL*. Markers not listed segregated 2:2 as expected. Complementation tests were performed to distinguish *his4* from *his1*.

DBA309: $\frac{a \text{ leu2-1 } SUP-RL1 \text{ trp1-1 } tyr7-1 \text{ can}^r}{\alpha \text{ leu2-1 } sup^+}$

DBH49: $a \text{ his4-280 } trp1-1 \text{ CAN}^s \text{ sup}^+$

DBA338: $\alpha \text{ ade1 his1 leu2-1 } trp1-1 \text{ tyr7-1 } can^r \frac{SUP-RL2}{sup^+}$

* A $\frac{SUP-RL}{sup^+}$ diploid will yield 2 live:2 dead spores per ascus.

† The mating-type locus is located on chromosome III. An extra third chromosome will yield spores of $\frac{a}{\alpha}$ genotype which will fail to mate.

DBA309 which had undergone a conversion or mutation from α/a to α/α . The *leu2-1* allele was donated by the aneuploid resulting in a trisomic configuration $-/-/+$. Rarely should this configuration result in aberrant tetrad ratios; only 2⁺:2⁻ tetrads were seen. Alternatively, it is possible that *leu2-1* is not included in the duplicated region.

Trisomy for the *SUP* locus was established by the finding that many tetrads (11 out of 22) contained more than two live spores. If the hybrid had contained only two copies of this locus, then each ascus should yield two live and two dead spores. When DBA 309 was mated with an *a* tester strain under conditions not requiring retention of the suppressor, the progeny all yielded 4 viable *sup*⁺ spores per ascus. This suggests that in the aneuploid DBA309, the *SUP* allele is present on the copy of chromosome III carrying the *a* mating-type allele. We conclude that DBA309 is aneuploid for chromosome III and that the *SUP-RL1* locus resides on this chromosome.

A recessive lethal amber suppressor not on chromosome III: Strain DBA338 was isolated by the random spore technique from *SUP* diploid DBD341 in the same manner as strain DBA309. Strain DBA338 differed from DBA309 in that it always displayed the α mating type. *Sup*⁺ segregants occurred at a frequency of about 1% in vegetative growth (Table 3).

In tetrad analysis of crosses between DBA338 and an *a trp1-1 tyr7-1* tester strain, equal numbers of *a* and α spores appeared, and no non-mating spores were detected. Table 4 contrasts results found in similar crosses of DBA309 and DBA338 to tester haploid strains.

TABLE 3

Segregation of SUP and mating type in aneuploid strains

Phenotype	Inferred genotype	Percentage of colonies which grew on rich plates	
		DBA309 (<i>SUP-RL1</i>)	DBA338 (<i>SUP-RL2</i>)
' α ' <i>Trp⁻Tyr⁻</i>	α <i>trp1-1 tyr7-1 sup⁺</i>	0.4	0.8
non-mater <i>Trp⁺ Tyr⁺</i>	<u>α <i>trp1-1 tyr7-1 SUP-RL</i></u> α <i>sup⁺</i>	99.6	0
non-mater <i>Trp⁻ Tyr⁻</i>	<u>α <i>trp1-1 tyr7-1 sup⁺</i></u> α	0	0
' α ' <i>Trp⁺Tyr⁺</i>	α <i>trp1-1 tyr7-1 SUP-RL</i> <i>sup⁺</i>	0	99.2

Strains DBA309 and DBA338 were grown to stationary phase in YEP-glucose, a rich medium which does not select for maintenance of suppressors. Dilutions were plated on YEP-glucose plates for single colonies. When grown up, these colonies were tested by replica plating for their ability to grow in the absence of tryptophan, tyrosine, and phenylalanine and for mating with standard α and α haploid tester strains.

Crosses to strain DBH49 (*his4-280*) confirmed that DBA338 is not disomic for chromosome III (Table 2). Of 22 asci with more than two viable spores, none gave aberrant tetrad ratios for the *his4* locus. This strongly suggests that the suppressor in DBA338 is not on chromosome III and thus not identical to the one present in DBA309.

TABLE 4

Analysis of spore progeny from crosses of aneuploids to tester haploid strains

	DBA338 \times DBH299	DBA309 \times DBH87
Number of tetrads	15	9
<i>ade⁺:ade1</i>	21:16	14:14
<i>his⁺:his⁻</i>	13:24*	15:13
<i>leu⁺:leu2-1</i>	0:37	17:11
<i>lys⁺:lys1-1</i>	20:17	—
<i>met⁺:met8-1</i>	31:6†	—
<i>CAN^s:can^r</i>	14:23	14:14
<i>sup⁺:SUP-RL</i>	23:14	18:10
α : α / α	16:21:0	12:3:10**

DBA338: α *ade1 his1 leu2-1 trp1-1 tyr7-1 can^r SUP-RL2*
sup⁺

DBH299: α *his4-580 leu2-1 lys1-1 trp1-1 tyr7-1 met8-1 CAN^s sup⁺*

DBA309: α *leu2-1 SUP-RL1* *trp1-1 tyr7-1 can^r*
 α *leu2-1 sup⁺*

DBH87: α *ade1 his1 trp1-1 tyr7-1 CAN^s sup⁺*

* Two *his⁻* markers are segregating.

† Excess of prototrophs not understood; however, commercial preparations of leucine are often contaminated with methionine.

** Three prototrophic spores: mating-type determination not performed.

DISCUSSION

We have isolated recessive lethal amber suppressor mutations in diploid strains of yeast. The frequency of this type of mutation is very high; in fact, a majority of the suppressors isolated in diploids (using reversion of two different pairs of amber mutations) were recessive lethals. At present, the number of loci capable of mutation to recessive lethal suppressor is unknown. On the basis of segregation of suppressor-bearing aneuploids, it is clear that at least two such loci exist. One locus is on chromosome III while another, as yet unmapped, is not on this chromosome.

Isolation of nonsense suppressors in yeast has heretofore been achieved almost exclusively in haploid strains. The best-studied group, the eight class I set 1 ochre suppressors, inserts tyrosine into protein (GILMORE, STEWART and SHERMAN 1971). A common interpretation of this finding is that eight redundant genes exist for the coding of tRNA^{Tyr} in *Saccharomyces cerevisiae*, and that each of the suppressors is an alteration in coding specificity at one of these genes. There is proof that tyrosine-inserting amber suppressors in *E. coli* are alterations in one of several redundant tRNA^{Tyr} genes (RUSSELL *et al.* 1970; SQUIRES *et al.* 1973); however, there is no direct evidence that the tyrosine-inserting suppressors in yeast are alterations in tRNA.

Since the suppressors we have isolated are recessive lethals, the wild-type form of the suppressor locus is essential. If these loci code for tRNA, then the tRNA may either be a unique coding species or carry out another vital function possibly unrelated to protein synthesis.

As one example of an alteration of a unique coding species, SOLL and BERG (1969) and MILLER and ROTH (1971) described the isolation of recessive lethal suppressors in merodiploids of *E. coli* and *S. typhimurium*, respectively. In *E. coli*, the mutation involved a single base change in the anticodon of the tryptophan tRNA altering codon recognition from UGG to UAG and simultaneously causing aminoacylation of glutamine instead of tryptophan (SOLL 1974; YANIV *et al.* 1974). Since *E. coli* is known to have only one gene coding for tRNA^{Trp} (HIRSH 1971), the loss of the ability to translate UGG as tryptophan is believed responsible for the recessive lethality.

By analogy to *E. coli*, one type of recessive lethal suppressor in *S. cerevisiae* could be an altered tRNA^{Trp}. Yeast appears to have a single species of this tRNA (two forms exist which differ only in that one U is modified to ψ) (KEITH *et al.* 1971), although the number of genes which code for tRNA^{Trp} is unknown.

Another explanation for the recessive lethality of these amber suppressor mutations could involve the efficiency of suppression. The suppressors might be so efficient in substituting an amino acid at the site of an amber codon that the physiology of the haploid cell is disrupted, resulting in failure to grow. In this case, it would not be the absence of an essential function but the oversuppression which is lethal to the haploid cell. Some effect on cell growth when suppression efficiency is enhanced was suggested by GILMORE (1967). By generating a very efficient suppressor mutation, it can be imagined that a diploid could be produced

with a recessive lethal phenotype, yielding two live spores and two dead spores. On the oversuppression hypothesis, if the suppressor mutations are in the tRNA genes, the tRNAs need not be unique coding species represented by a single gene copy in the genome of yeast.

One argument in favor of the essential tRNA species idea is the generation of aneuploids (*SUP-RL/sup⁺*); these would not be anticipated by the oversuppression model.

Cox (1971) has described a non-mendelian factor, *psi*, which can bestow the recessive lethal phenotype on some yeast suppressors. The eight class I set 1 suppressors are expressed in *psi⁻* cells, but are lethal in *psi⁺* cells. Cox proposed that the *psi* factor, in the presence of these ochre suppressors, enhanced suppression efficiency to a level lethal to the cell. We do not believe that the recessive lethality of the suppressors described here is related to the *psi* factor; by their genealogy all of our strains lack it, and recombinants with ordinary class I set 1 suppressors are viable.

HAWTHORNE and LEUPOLD (1974) mention the isolation of a recessive lethal suppressor, *SUP61*, from a diploid strain of yeast. All asci analyzed gave two live spores per ascus. The spores carrying the recessive lethal suppressor mutation were rescued by spore mating shortly after germination and the suppressor recovered. Analysis was performed on a trisome. *SUP61* has been mapped on chromosome III (MORTIMER and HAWTHORNE 1973), about 40 cM distal to the mating-type locus. The suppressor (*SUP-RL1*) in DBA309 could well be identical to *SUP61*.

Experiments to determine amino acid insertion and precise mapping for both suppressors are in progress.

We thank DR. FRED SHERMAN, DR. MIRIAM M. SUSSKIND and GREGORY PAHEL for stimulating discussions. This work was supported by a grant from the American Cancer Society (#VC18C). MCB is an NIH predoctoral trainee under grant #5-G01-GM00602-15; during completion of this work, LS was a fellow of the Medical Foundation, Boston, under the auspices of the Godfrey M. Hyams Trust; DB holds a Career Development Award from NIH, #5-K04-GM70325-02.

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Corresponding Editor: G. R. FINK