REVERSION FROM SUPPRESSION TO NONSUPPRESSION IN *SUQ5* [*psi*+] STRAINS OF YEAST: THE CLASSIFICATION OF MUTATIONS

B. S. COX, M. F. TUITE* AND C. J. MUNDY†

Botany School, South Parks Road, Oxford, U.K.

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ABSTRACT

Reversion from the suppressed to nonsuppressed phenotype in strains of genotype *SUQ5* [*psi*+] *ade2-1 his5-2 lys1-1 can1-100 ura3-1* has been induced by treatment with ethyl methanesulphonate, nitrosoguanidine or UV (254 nm) light. Spontaneously occurring revertants have also been selected by two different methods. Reversion has been shown to occur through a variety of nuclear mutations and through mutation of [*psi*+] to [*psi*−]. Nuclear mutations included back-mutation of *SUQ5*, antisuppressor mutations that were recessive, semi-dominant or dominant, and dominant or recessive mutations of genes required for the maintenance of the [*psi*+] factor. Complementation tests by which the various kinds of mutations could be distinguished from one another were designed. The spectra of spontaneously occurring and induced mutations have been described.

IN certain strains of yeast, the suppression of ochre (UAA) mutations depends upon two genetic factors, one nuclear and the other extrachromosomally inherited (Cox 1965). The nuclear determinant, *SUQ5*, is a gene coding for a serine-inserting tRNA (LIEBMAN, STEWART and SHERMAN 1975; CAPPECHI, HUGHES and WAHL 1975). The extrachromosomal element, [*psi*], has not been identified with any particle or component of the cell, nor is its mode of action understood. Its effect is to increase the efficiency of suppression so that, for example, read-through of the ochre codon in the mutant *cyc1-2* is 15 times more likely in an *SUQ5* [*psi*+] than an *SUQ5* [*psi*−] *strain* (LIEBMAN, STEWART and SHERMAN 1975).

We have been studying the nature of this two-component system by means of agents that affect suppression. The work has included studies of agents that block or enhance suppression phenotypically, of agents that cause mutations in the genetic system and of genes that affect the expression or inheritance of the genetic components of the system. In this paper, we describe the range of mutations that block nonsense suppression in *SUQ5* [*psi*+] strains and the methods we have used for analyzing them.

* Present address: Department of Biochemistry, School of Medicine, University of California, Irvine, California 92717.
† Present address: Chemical Carcinogenesis Division, Institute of Cancer Research, Pollards Wood Research Station, Nightingales Lane, Chalfont St. Giles, Bucks, U.K.

We anticipated four types of revertants from a suppressed to a nonsuppressed phenotype. First, there would be reversions of the suppressor gene, *SUQ5*, to wild-type *suq5*+. Second, there would be reversions of the extrachromosomal determinant from [*psi*+] to [*psi*-]. Third, we expected antisuppressor mutations of the type described by McCREADY and COX (1973). The fourth type would be the kind of mutant described by YOUNG and COX (1971) and McCREADY, COX and McLAUGHLIN (1977) termed "R" or "PNM." These appear partly or completely to block the inheritance of the [*psi*+] factor.

Our objective was to show that these types of reversions do occur and, in order to make genetic analysis of the system feasible, to devise a set of tests that are easy to carry out and that can distinguish them.

Most of the ochre mutations in yeast are auxotrophic, and suppression yields a prototroph. Consequently, reversions from a suppressed to a nonsuppressed phenotype are analagous to "forward" mutations. In general, it is not possible to "select" such phenotypes from a large population of nonmutant cells. We describe here two systems that may be used to select spontaneously arising nonsuppressed revertants. They are compared with a nonselective system of suppressed revertants. They are compared with a nonselective system of "screening" for revertants, which is suitable when the rate of reversion can be enhanced by mutagens.

We first describe the genetic analysis of 44 nonsuppressed revertants recovered from two suppressed strains, 467/4a and 507.4/2b. These comprise all the types of revertant we expected to find, together with some unexpected types. One of these is a nuclear mutation that, in its complementation interaction and genetics, may be indistinguishable from a [*psi*-] reversion; we also describe and justify the test we have devised to discriminate between the two. Finally, we compare the spectra of spontaneous reversions obtained from the two selection methods described and of the revertants obtained by mutagenic treatment followed by nonselective screening.

MATERIALS AND METHODS

Strains: Reversions have been obtained from and studied in the following strains. The abbreviations for the genetic markers are explained in the supplement to the Yeast Microbial Genetics Bulletin No. 31 (VON BORSTEL 1969). A superscript "o" indicates an ochre mutation.

467/4a: α, *ade2-1*^{o}, *his5-2*^{o}, *lys1-1*^{o}, *can1-100*^{o}, *ura3-1*, *SUQ5*, [*psi*+]

507-4/2b: α, *ade2-1*^{o}, *his5-2*^{o}, *lys1-1*^{o}, *can1-100*^{o}, *ura3-1*, *SUQ5*, [*psi*+].

These were derived from strains described earlier (COX 1965) and from strains obtained from the Yeast Stock Center, Berkeley, namely, X2314-8c = α, *ade2-1*^{o}, *his5-2*^{o}, *lys1-1*^{o}, *trp5-48*^{o}, *can1-100*^{a}, *ura3-1*, *SUP2*, and X2315-14C = the same, but *SUP3*. These were kindly provided by R. K. MORTIMER.

Strains for determining the genotypes of the revertants by genetic analysis were also derived from these sources. The *kar*- mutation was introduced from strain JC8 = **a**, *leu1*, *kar1*, provided by G. R. FINK.

A strain of genotype **a**, *ade2-1*, *his5-2*, *can1-100*, *leu1*, *kar1*, [*psi*+] (MT 152/3c) was derived from a cross between JC8 and MT191/8c (α, *ade2-1*, *his5-2*, *can1-100*, *KAR*+ [*psi*+]). The presence of the *kar1* mutation in the *ade2-1* (red) segregants was determined by cross-

streaking with **a** or *α ade1* testers. *kar1* strains yielded only rare complementing diploids in the mixed culture and were easily distinguishable from the *KAR+ ade2-1* segregants that gave a massive complementation reaction.

Media: The standard growth medium was YEPD (1% yeast extract, 1% peptone, 2% dextrose) solidified with Oxoid agar. Complementation tests were done on YEP8%D (YEPD containing 8% dextrose), or YC, the yeast complete medium described by Cox and Bevan 1962.

The basal omission medium (YNB) was 0.67% Difco Yeast Nitrogen Base without amino acids, 2% dextrose, 1.5%Oxoid purified agar. Amino acids, adenine or uracil were added as required at 10 or 20 μg/ml.

Selection of canavanine-resistant mutants, or scoring of canavanine requirement, was done using YNB containing canavanine at 30 μg/ml.

Sporulation medium was that described by Hurst and Fogel (1964). In certain experiments, when we wished to stimulate sporulation immediately, the medium described by Pomper, Daniels and McKee (1954) was used.

Mutagenesis: Treatment with EMS was done according to the protocol described by Lindegren *et al.* (1965). Cells in stationary phase were suspended in 3% v/v EMS in 2% glucose, 0.02 M phosphate buffer at pH 8.0 and incubated at 37° for 70 min with occasional shaking. They were diluted 100-fold into 6% sodium thiosulphate before further dilution in 0.85% saline and plating.

UV irradiation was carried out on stationary-phase cells suspended at a titer of approximately 10^7 cells/ml in 0.85% saline. 10 ml of suspension was placed in a 9 cm petri dish and irradiated with 254 nm UV (Unrau *et al.* 1973). Dilutions were made in 0.85% saline before plating. All operations were carried out in yellow light to avoid photoreactivation.

Treatment with N-Methyl-N-Nitro-N-Nitrosoguanidine (NTG) was as follows: A stock solution of NTG (Koch-Light) of 10 mg/ml in acetone was prepared immediately prior to use. Appropriate aliquots were added immediately to 10 ml of a sterile 0.2 M phosphate buffer (pH 7.0) in a 50 ml flask covered by silver paper, and containing cells at a titer of 10^5–10^6/ml. After shaking for 45 min at either 23° or 28°, 200 μl of the suspension was added to 9.8 ml of a 6% (w/v) sterile sodium thiosulphate solution and incubated at 28° for 10 min to inactivate the NTG. The cell suspension was then diluted in sterile saline and spread onto YC plates.

Genetic analysis: Strains of opposite mating type were mated by mixing them together on the surface of a YEPD plate and incubating for 4 hr at 28° to obtain diploids. Zygotes were picked by micromanipulation and grown into individual clones. Tetrads were obtained by transferring such clones, or the random-mating mixtures of cells, to sporulation medium and incubating for 4 days at 25°. Tetrad analysis was performed by digesting asci with a solution of 5% v/v glusulase in distilled water and separating the spores by micromanipulation. Glusulase was obtained from Endo Laboratories, Inc., N.J. The micromanipulator used was a Saunders-Singer Mark III.

Complementation tests exploited the red pigment accumulated by *ade2* mutants of yeast. All the strains used carried the ochre allele *ade2-1*. Suppressed strains were prototrophic in phenotype and white in color. Nonsuppressed revertants accumulated the pigment and were red. The color was deeper when colonies were grown on YEP8%D, or on YC. On these media, adenine-requiring petities were also red. To determine whether diploids formed from 2 nonsuppressed haploid strains were suppressed, streaks of the 2 haploids were made across each other on YEP8%D or YC. The plate was incubated and, where the streaks crossed, diploid clones grew and their color was observed (Figure 2). The cross-streaks were made either with "dry" inocula or by making a suspension of cells at a titer of about 10^7 cells/ml and cross-streaking these. The vertical streaks were allowed to dry before making the cross-streaks. The difference between the two methods is largely aesthetic, although when complementation is weak, it is more easily observed through the use of the second technique. It was usual to employ 2 tester strains of the type *ade2-1*, *SUQ5*, [*psi*⁻], to reduce ambiguity arising from their high reversion frequency.

RESULTS

Isolation of mutants

(a) *Spontaneous mutation:* Reversion to an unsuppressed phenotype in a strain of genotype *ade2–1 SUQ5* [*psi*+] results in a clone that is red in color due to the accumulation of a red pigment of cells requiring adenine for growth. Such revertants are rarely observed. We used two methods of selecting nonsuppressed revertants of the strain 507.4/2b = α, *ade2–1*, *his5–2*, *lys1–1*, *can1–100*, *ura3–1*, *SUQ5*, [*psi*+]. The first made use of the ochre mutation, *can1–100*, conferring canavanine resistance. 507.4/2b is canavanine sensitive, since the *can1–100* mutation it contans is suppressed. Canavanine-resistant clones were selected by plating cells on supplemented YNB containing 30 μg/ml canavanine. Canavanine-resistant revertants arise either by second-site mutations in the canavanine locus or by mutation abolishing suppression. The latter grow as red colonies, since the *ade2–1* locus is also no longer suppressed.

We have also observed that many *SUQ5* [*psi*+] strains are cold sensitive in that they grow extremely slowly at low temperatures. The doubling time of a nonsuppressed strain is less than half that of a suppressed strain at 12°. This can be exploited to select nonsuppressed revertants. A lawn of cells of genotype *SUQ5* [*psi*+] *ade2–1* spread on *YEPD* and incubated at 12° soon acquires several faster-growing red papillae. We shall describe our investigation of this phenomenon in more detail elsewhere. Here, it is sufficient to say that we have been unable to demonstrate that growth at low temperatures *induces* mutation, either nuclear or cytoplasmic, and the revertants that arise on these plates are probably simply selected from a preexisting population of mutants by their ability to grow faster in the cold.

We isolated "jackpot-free" random samples of revertants from 507.4/2b by each of these methods. Random samples were obtained by separately resuspending individual colonies from a plating of 507.4/2b and respreading each suspension on a single plate to select revertants. One red revertant was picked from each plate.

(b) *Induced revertants:* Revertants were also obtained after treatment of suppressed strains with mutagen. Three mutagens were used.

A suspension of 467/4a at a titre of 10^7 cells/ml. was treated with EMS as described in the METHODS section. The suspension was plated on YNB supplemented with canavanine, adenine, histidine, lysine, tryptophan and uracil. Red revertants were picked for analysis.

507.4/2b was treated with either nitrosoguanidine or with UV as described in the METHODS section. Treated cells were plated on YC medium and revertant clones were identified by their red color after growing at 28° for four days. Picking red colonies on YC introduces two classes of mutation that do not affect suppression. These are mutations in the *ADE1* locus or second-site mutations in the *ade2* locus. These were distinguished from the other classes by the fact that they were still *HIS*+ *LYS*+ and canavanine sensitive and from each other by a com-

plementation test with a tester of genotype **a** *ade1*. This tester strain also demonstrated the rare occurrence of nonmating revertants.

Genetic analysis

A selection of revertants was chosen for detailed analysis. Initially, they were classified by their complementation pattern with two kinds of tester strains, namely: (1) SUQ5 [psi⁻] **a** *ade2–1 SUQ5* [*psi⁻*] (193/1b) and (2) $suq5^+$ [psi^+] **a** *ade2–1 his5–2, lys1–1 can 1–100* [psi^+] (466/6c or 465/2a).

These "tester" strains were, like the revertants, unsuppressed and red in color because they were lacking one or both of the components of the suppressor system.

These tests divide the revertants into four classes as follows: (I) complementing both (1) and (2); (II) complementing neither; (III) complementing (1) but not (2); and (IV) complementing (2) but not (1) (Table 1).

Revertants in each of these classes were analyzed further by dissecting tetrads from the diploids formed by crosses with the second type of tester strain, 465/2a or 466/6c = *ade2–1* $suq5^+$ [psi^+]. The analyses are considered in turn.

Class I revertants complemented both [*psi⁻*] and [$suq5^+$] strains. Therefore, they still carried both [psi^+] and *SUQ5* determinants. The loss of the suppressed phenotype was likely, therefore, to be due to recessive antisuppressor (*asu*) mutations of the type described by McCready and Cox (1973). These revertants, when crossed with 466/6c or 465/2a, gave diploids of the genotype:

$$[psi^+] \quad \frac{asu}{+} \quad \frac{SUQ5}{+} \qquad \frac{ade2\text{–}1}{ade2\text{–}1} \quad \frac{his5\text{–}2}{his5\text{–}2} \quad \frac{lys1\text{–}1}{lys1\text{–}1} \quad \frac{can1\text{–}100}{can1\text{–}100} \quad \frac{ura3\text{–}1}{+}.$$

Because both the antisuppressor and the suppressor were heterozygous, tetrads yielded a mixture of segregants of suppressed:nonsuppressed spores of 2:2 (nonparental ditype). 1:3 (tetratype) and 0:4 (parental ditype). (Table 1, lines 1–11).

Class II revertants complement neither [*psi⁻*] nor $suq5^+$ tester strains. They have, therefore, either acquired mutations simultaneously in both the [psi^+] factor and the *SUQ5* gene or a single dominant mutation abolishing suppression. Tetrads from 12 revertants were analyzed. They fell into two classes (Table 1, line 12–23). Six of the revertants (7 and 10 from 467/4a and II–1, 2, 3, 5 and 6 from 507.4/2b) segregated spores that were suppressed. Therefore, they still carried the suppressor, *SUQ5*. We have classified these as being due to dominant antisuppressor mutations.

The other five revertants behaved differently. Tetrads derived from the diploids formed with 466/6c or 465/2a ($SUQ5^+$ [psi^+]), (tester No. 2) all yielded four unsuppressed cultures, although there was in each set a segregation of 2 slowgrowers: 2 nongrowers on the omission media lacking histidine or lysine.

The complementation patterns of all the progeny from revertants 15, 16 and 19 were tested. They were either of Class II (noncomplementing) or Class IV

TABLE 1

The genetic analysis of unsuppressed revertants of 467/4a and 507.4/2b, both α ade2–1° his5–2° lys1–1° trp5–48° can1–100° ura3–1 SUQ5 [psi+]

	Strain	Revertant no.	Color of diploid with *suq5*+[*psi*+]	*SUQ5*[*psi*-]	Segregation in tetrads of *ADE*+:*ade*- 4:0	3:1	2:2	1:3	0:4	Nos. of [*psi*+]	[*psi*-]	Genotype
Class I.												
	467/4a											
1.		1	W	W	0	0	1	4	0			
2.		4	W	W	0	0	0	4	1			
3.		9	W	W	0	0	0	4	1			
4.		11	W	W	0	0	0	2	3			
5.		14	W	W	0	0	2	1	1			*asu*
	507.4/2b											
6.		I—1	W	W	0	0	3	5	1			
7.		I—2	W	W	0	0	4	5	1			
8.		I—3	W	W	0	0	2	7	1			
9.		I—4	W	W	0	0	1	7	2			
10.		I—5	W	W	0	0	2	6	2			
11.		I—6	W	W	0	0	6	0	1			
Class II.												
	467/4a											
12.		8	R	R	0	0	0	0	5			
13.		15	R	R	0	0	0	0	4	0	16	
14.		16	R	R	0	0	0	0	5	0	20	*PNM*
15.		19	R	R	0	0	0	0	4	0	20	
	507.4/2b											
16.		II—4	R	R	0	0	0	0	12			
	467/4a											
17.		7	R	R	Random spores: 1 *ADE*+, 7 *ade*-							
18.		10	R	R	0	0	0	3	2			

TABLE 1—Continued

Strain		Revertant no.	Color of diploid with $suq5^+$[*psi*$^+$]	$SUQ5$[*psi*$^-$]	Segregation in tetrads of ADE^+:ade^- 4:0	3:1	2:2	1:3	0:4	Nos. of [*psi*$^+$]	[*psi*$^-$]	Genotype
	507.4/2b											
19.		II — 1	R	R	0	0	0	1	9			
20.		II — 2	R	R	0	0	0	3	8			
21.		II — 3	R	R	0	0	0	3	7			*ASU*
22.		II — 5	R	R	0	0	0	5	4			
23.		II — 6	R	R	0	0	0	1	7			
Class III.												
	467/4a											
24.		3	R	W	0	0	0	0	5	13	7	
25.		6	R	W	0	0	0	0	10	38	2	
26.		12	R	W	0	0	0	0	4	11	5	
27.		17	R	W	0	0	0	0	4	13	3	*suq5*+
	507.4/2b											
28.		III — 1	R	W	0	0	0	0	8			
29.		III — 2	R	W	0	0	0	0	8			
30.		III — 3	R	W	0	0	0	0	10			
31.		III — 4	R	W	0	0	0	0	10			
	467/4a											
32.		5	R	W	0	0	0	2	3			*Asu*
	507.4/2b											
33.		III — 5	R	W	0	0	2	7	2			
Class IV.												
	467/4a											
34.		2	W	R	0	0	0	0	5	1	19	
35.		13	W	R	0	0	0	1	3	3	13	
36.		18	W	R	0	0	1	3	0	8	8	

TABLE 1—Continued

	Strain	Revertant no.	Color of diploid with		Segregation in tetrads of $ADE^+:ade^-$					Nos. of		Genotype
			$suq5^+[psi^+]$	$SUQ5[psi^-]$	4:0	3:1	2:2	1:3	0:4	$[psi^+]$	$[psi^-]$	
	507.4/2b											
37.		IV—1	W	R	0	0	2	2	2			*pnm*
38.		IV—2	W	R	0	0	1	8	2			
39.		IV—3	W	R	0	0	1	4	2			
40.		IV—5	W	R	0	0	3	4	1			
41.		IV—6	W	R	0	0	1	5	1			
42.		IV—4	W	R	0	0	6	0	0			
43.		Me—1	W	R	0	0	11	0	0			$[psi^-]$
44.		Me—3	W	R	0	0	15	0	0			

The tetrads were derived from diploids formed by crossing the revertants with a tester strain of type 2: **a** *ade2-1 his5-2 lys1-1 suq5*$^+$ $[psi^+]$.

(complementing *sup5*$^+$ [*psi*$^+$]. The segregation patterns among 13 tetrads were:

4 II : 0 IV—4; 3 II : 1 IV—8 and 2 II : 2IV—1 .

The occurrence of spore clones that complement *suq5*$^+$ [*psi*$^+$] strains indicated that these revertants still contained *SUQ5*. This was also suggested by the segregation of two partially suppressed spores in each tetrad.

The failure of any segregant to complement *SUQ5* [*psi*$^-$] strains indicated that all the segregants were [*psi*$^-$]. Since they were derived from diploids in which one of the parents (466/6c) was [*psi*$^+$], it follows that the revertants all contained a mutation that rendered them [*psi*$^-$]. Since the parent diploids were heterozygous for *SUQ5* (but unsuppressed) and segregated only [*psi*$^-$] offspring, they too were [*psi*$^-$]. Hence, the mutation inactivating [*psi*] in each revertant was dominant. This is characteristic of an R or PNM mutation (YOUNG and COX 1971).

The Mendelian segregation of such a mutation was demonstrated in two stages. A tetrad from revertant 19 × 466/6c was chosen. None of the spores complemented *suq5*$^+$ [*psi*$^+$] tester strains. It was, therefore, expected to have two spore clones of constitution *suq5*$^+$, *pnm*$^+$, [*psi*$^-$] and two *SUQ5, PNM,* [*psi*$^-$]. Single zygotes were isolated from mixtures of the spore clones with the *SUQ5* [*psi*$^+$] strains 467/4a (α) or 466/5c (**a**). Zygote clones from two of the matings were white and two were red. The phenotypes and complementation patterns of clones from this tetrad are shown in Table 2, columns A through D, and the genotypes inferred from these phenotypes are shown in column E.

The diploids marked SS in this table should be homozygous *SUQ5/SUQ5*. Asci from these diploids were dissected. The behavior and phenotypes of the segregants of these tetrads are illustrated in Table 2, columns F and G. In both sets of tetrads, there was a 2:2 segregation of the ability to complement red strains of genotype *suq5*$^+$ [*psi*$^+$]. This confirms that the diploids were homozygous *SUQ5/SUQ5*, and demonstrates the Mendelian segregation of the presence (preventing complementation) or absence (allowing it) of the *PNM* gene. A tetrad from revertant 16 × 466/6c was analyzed in the same way, yielding similar results.

Class III revertants complemented [*psi*$^-$], but not *suq5*$^+$ tester strains. This indicated that they were still [*psi*$^+$], but were, apparently, revertant at the suppressor locus. Such revertants would form diploids with tester 2, of genotype:

$$[psi^+]\ \frac{ade2\text{-}1}{ade2\text{-}1}\ \frac{his5\text{-}2}{his5\text{-}2}\ \frac{lys1\text{-}1}{lys1\text{-}1}\ \frac{can1\text{-}100}{can1\text{-}100}\ \frac{ura3\text{-}1}{+}$$

and these diploids would, of course, segregate no suppressed spore clones in tetrads.

Eight of the Class III revertants followed this pattern (Table 1, lines 24–31). The other two revertants segregated suppressed spore clones and were, therefore, still *SUQ5* (Table 1, lines 32 and 33). Since they complemented the [*psi*$^-$] tester, they were also still [*psi*$^+$]. It follows that they were unsuppressed because

TABLE 2

Segregation of phenotypes in a tetrad derived from revertant 19 × 466/6c (ade2-1 [psi+]) *and in a second generation of tetrads from matings with 467/4a and 466/5c* (ade2-1 SUQ5 [psi+])

	Growth on *OM-his* (A)	*Color of diploids formed with* *suq5*+[*psi*+] (B)	*SUQ5*[*psi*−] (C)	*SUQ5*[*psi*+] (D)	Genotype (E)	Tetrads from (D) (F)	Segregation of complementation of + [*psi*+] (G)
474.19/1a	slow	red	red	red^{SS}	*SUQ5 PNM*	9 × OX:4R	8 × 2+:2− 1 × 1+:2−
474.19/1b	slow	red	red	red^{SS}	*SUQ5 PNM*	10 × OW:4R	10 × 2+:2−
474.19/1c	0	red	red	white	+ +[*psi*−]	—	—
474.19/1d	0	red	red	white	+ +[*psi*−]	—	—

of antisuppressor mutations unlinked to the *SUQ5* locus. The failure to complement *suq5*$^+$ showed that the antisuppressor mutation was not fully recessive. It inhibited suppression when *SUQ5* was heterozygous *SUQ5*/+ but not when it was homozygous *SUQ5/SUQ5*. The revertants, therefore, also failed to complement a strain of genotype *suq5*$^+$ [*psi*$^-$].

Class IV revertants complemented the *suq5*$^+$ [*psi*$^+$] tester strains. Therefore, they still carried *SUQ5*; however, they failed to complement the *SUQ5* [*psi*$^-$] tester and were apparently [*psi*$^-$].

Diploids formed between [*psi*$^-$] revertants and testers of type 2, *suq5*$^+$ [*psi*$^+$] would have the genotype:

$$[psi^+] \quad \frac{ade2\text{–}1}{ade2\text{–}1} \quad \frac{his5\text{–}2}{his5\text{–}2} \quad \frac{lys1\text{–}1}{lys1\text{–}1} \quad \frac{can1\text{–}100}{can1\text{–}100} \quad \frac{ura3\text{–}1}{+} \quad \frac{SUQ5}{+} .$$

They would segregate 2 suppressed:2 nonsuppressed spores in every tetrad and all segregants would be [*psi*$^+$]. The diploids formed by eight revertants (Table 1, lines 34–41) yielded an excess of unsuppressed spore cultures. From one of them, revertant 2, no suppressed spore cultures segregated at all. All the unsuppressed progeny from three revertants were crossed to see if they complemented strains of genotype *SUQ5* [*psi*$^-$]. The results indicated that some of the segregants in each cross were [*psi*$^+$] and some [*psi*$^-$]. There were no more than two [*psi*$^+$] segregants in each tetrad and, in tetrads from diploids 2 and 13, most of the segregants were [*psi*$^-$], (Table 1, lines 34–36).

These segregations indicated the presence, in the revertant strains, of a mutation that converted the cells to [*psi*$^-$] but was recessive, since the diploid formed with a *suq5*$^+$ [*psi*$^+$] strain was suppressed and, therefore, [*psi*$^+$]. The mutation also interfered to a greater or lesser extent with the segregation of the [*psi*$^+$] determinant at meiosis. The 2 [*psi*$^+$]: 2 [*psi*$^-$] segregation observed in progeny of revertant 18 suggests that the mutation is chromosomal. It is thus analogous to the *pet18* mutation controlling the segregation of [*rho*] and the *mak* mutations controlling the segregation of the killer plasmid (LEIBOWITZ and WICKNER 1978; WICKNER and LEIBOWITZ 1976). Apart from the fact that it is recessive, it is similar to the *PNM* mutations already described. We have called it *pnm*.

The other three revertants of Class IV yielded the segregation pattern expected of [*psi*$^-$] mutations (Table 1, lines 42–44).

To summarize the results of the genetic analysis presented here, we have identified up to seven types of mutation that may occur to reduce or abolish suppression in strains of genotype SUQ5 [*psi*$^+$]. They are: (1) [*psi*$^-$], mutations of the [*psi*] factor; (2) *suq5*$^+$, reversions at the suppressor locus; (3) *asu*, recessive antisuppressors; (4) *Asu*, semi-dominant antisuppressors; (5) *ASU*, dominant antisuppressors; (6) *PNM*, dominant mutations converting [*psi*$^+$] to [*psi*$^-$]; and (7) *pnm*, recessive mutations converting [*psi*$^+$] to [*psi*$^-$].

These are more classes than could be distinguished by the simple complementation tests used. Their identification depended upon tetrad analysis of diploids formed by mating the revertants with strains of genotype *suq5*$^+$ [*psi*$^+$].

Clearly, for any studies concerning the mutation, maintenance or inheritance of the [*psi*] factor, it is important to design a simple method to distinguish between the cytoplasmic factor and any nuclear mutation that mimics it. In particular, the recessive *pnm* raises problems in designing a simple complementation test to distinguish nonsuppressed revertants from one another. The phenotypes of diploids formed with *pnm* mutants are identical to those formed with [*psi*⁻] strains. Only in the segregation of [*psi*⁺] after meiosis do they differ.

The kar *test for cytoplasmic revertants of* [psi⁺] *to* [psi⁻]

Recently, FINK and CONDE (1977) described the properties of a mutation in yeast that interferes with the process of mating. The *kar1* mutation blocks karyogamy of any nucleus in which it occurs, but in no way interferes with plasmogamy. Rarely, diploid nuclei are formed, so that meiosis and sporulation can be induced, thus making genetic analysis possible. The heterokaryotic cells formed by plasmogamy frequently segregate homokaryotic or haploid buds. If, in a mating involving a *kar1* mutant, the cytoplasmic genomes differ, the heterokaryon is also a heteroplasmon, as are any homokaryotic or haploid cells that segregate from it (WRIGHT and LEDERBERG 1957; JINKS 1963). This can be used to distinguish nuclear from cytoplasmic mutations leading to nonsuppression. Consider a nuclear mutation, let us say *pnm*⁻. The genotype of the revertant would be α *ade2–1 SUQ5 pmn* [*psi*⁻]. If this were mated with a strain of genotype **a** *ade2–1 suq5*⁺ *kar* [*psi*⁺] the heterokaryon would be [*psi*⁺] and would segregate haploids of either parental nuclear genotype. The **a** segregants would be, as was the **a** parent, *suq5*⁺ [*psi*⁺], but the α segregants, because of the *pnm*⁻ mutation, would become [*psi*⁻] and remain unsuppressed. Apart from the few heterokaryon and diploid cells, no suppressed cells segregate. Indeed, an α nucleus carrying a mutation preventing suppression remains unsuppressed on segregating from the heteroplasmon. However, if the α parent is [*psi*⁻], but otherwise unchanged, the α segregants from a mating with the *kar* strain will carry the [*psi*⁺] factor introduced by the mating type **a** parent and be suppressed. The test is illustrated in Figure 1.

The *kar* strain MT152/3c described in MATERIALS AND METHODS was crossed with nonsuppressed revertants of 507.4/2b (α *ade2–1, his5–2, lys1–1, can1–100, ura3–1, SUQ5* [*psi*⁺]). Two of these were *pnm* mutants and two [*psi*⁻] mutants (Table 1, lines 37, 38, 43 and 44). The *kar* strain was also crossed with a known [*psi*⁻] strain, 468/1d, (α *SUQ5* [*psi*⁻] *ade2–1 his5–2 can1–100 ura3–1*). Several individual zygotes were picked from the mating mixtures and grown into colonies.

Heterokaryotic zygotes are expected to give rise to mixed clones, since they segregate haploids with nuclei of both parental types. However, it was found that, although in all matings some zygote colonies were of mixed types, as expected, others were pure clones. The types and numbers are shown in Table 3. It is likely that the pure clones arose from zygotes that were either diploid or segregated only one of the haploid parental types. (FINK and CONDE 1977). The pure zygote colonies were not analyzed further except to establish that the white

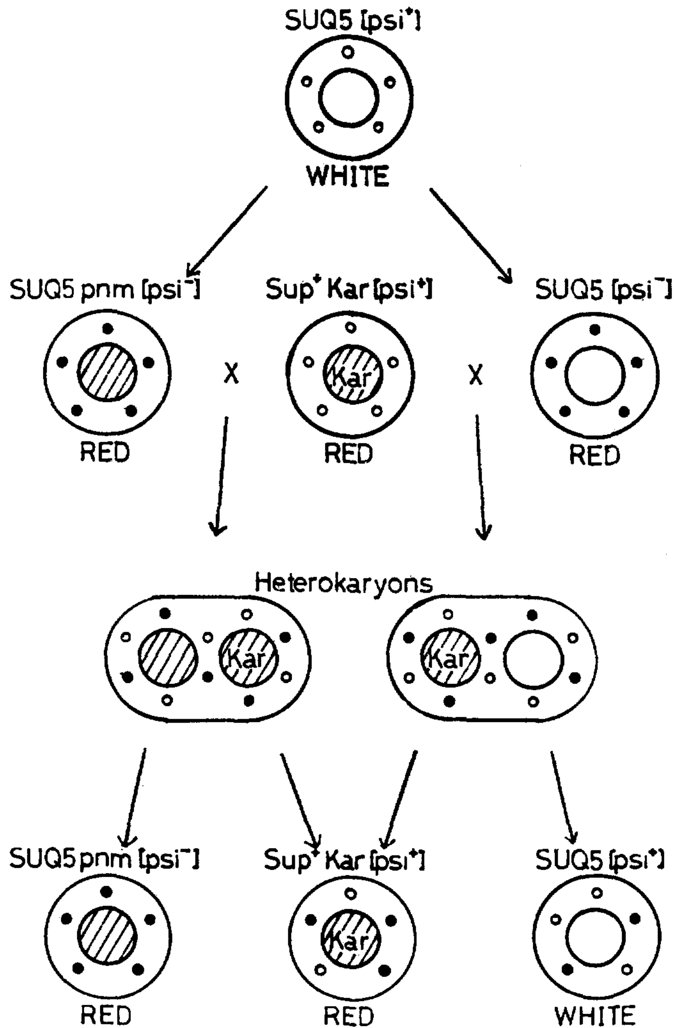

FIGURE 1.—An illustration of the "*kar*" test to distinguish nuclear mutations from cytoplasmic mutations. The diagrams show how a nonsuppressing *Kar* [*psi*+] strain can complement an unsuppressed cytoplasmic [*psi*−] mutant, but not a mutant in which failure of suppression is due to a nuclear mutation. ⊘ A nucleus of a nonsuppression genotype. ○ A nucleus of a suppression genotype. ° [*psi*+]. • [*psi*−].

colonies from the [*psi*−] crosses were α LEU^+, *ura*− and, therefore, inherited their nuclei from the unsuppressed, [*psi*−] parent.

The types of cells expected to segregate from the "mixed" heterokaryotic colonies are shown in Table 4. A comparison with Table 3 suggests that so few diploids segregated from these matings that white clones or sectors were seldom apparent among the progeny of *kar* × *pnm* parents. However, the progeny of *kar* × [*psi*−] parents may segregate as many as 50% white clones, since when-

TABLE 3

The phenotypes of clones derived from zygotes of pnm⁻ *and* [psi⁻] *strains mated with MT 152/3c* = *a* [psi+] kar1 ade2–1 his5–2 can1–100 leu–1

Strain (see Table 1)	Genotype	Phenotype of zygotic clones			
		Red	White	Red & pink & white	Red & pink
507-Me-1	[*psi⁻*]	2	3	4	0
507-Me-3	[*psi⁻*]	2	2	4	0
468/1d	[*psi⁻*]	4	6	9	0
507 *pnm*-1	*pnm*	2	0	0	2
507 *pnm*-2	*pnm*	2	0	0	2

ever the α parent nuclei segregate, carrying *SUQ5*, they inherit the [*psi*+] factor from the mixed cytoplasm of the zygote.

This was confirmed by taking one "mixed" clone from each cross and replating each one on either YC or omission medium lacking leucine. The numbers of colonies of different types recovered are recorded in Table 5.

We describe the [*psi⁻*] × *kar* matings first (lines 1, 2, 3, 6 and 7). Red colonies segregating from [*psi⁻*] × **a** *kar suq5+ leu1* heterokaryotes were recovered after plating on YC, but not leucineless medium. They all had, therefore, inherited nuclei from the *leu⁻ kar* parent. The same heterokaryon segregated many white colonies that grew on both YC plates and leucineless plates; 129 of these were tested and found to be α *ura⁻ LEU+*. They were, therefore, clones that had inherited nuclei from the unsuppressed [*psi⁻*] parent together with [*psi*+] from the *kar* parent. This is clear confirmation of the cytoplasmic location of the [*psi*] factor. The remaining colonies grew from heterokaryotic cells. They continued to segregate **a** *kar suq5+ leu⁻* cells even when growing on leucineless medium, as was shown by the red sectors that became apparent after replica plating to YC plates.

The zygote clones from the *pnm* × *kar* matings behaved differently. First, red clones segregated that grew on leucineless medium; these were clones that had

TABLE 4

The phenotypes expected of clones formed by matings of a [psi+] kar *strain with either* SUQ5 [psi⁻] *or a* SUQ5 pnm *strain*

	[*psi⁻*] *SUQ5* (α, *ade2–1*, *ura3–1*)	×	[*psi*+] *kar1* (**a**, *ade2–1*, *leu1*)	×	*pnm SUQ5* (α, *ade2–1*, *ura3–1*)
1. *Haploid:*	white, *ADE+* (α, *LEU+*, *ura⁻*)		red, *ade⁻* (**a**, *leu⁻*, *URA+*)		red, *ade⁻* (α, *LEU+*, *ura⁻*)
2. *Heterokaryon:*	pink + white + red				pink + red
3. *Rare Diploid:*			white, *ADE+* (2n, *LEU+*, *URA+*)		

TABLE 5

The phenotypes of colonies recloned from zygotic clones derived from three "cytoplasmic" psi⁻ *mutants (Me1, Me-3 and 468/1d) and two* pnm psi⁻ *mutants crossed to MT 152/3c* (**a** ade2-1 leu1 kar1 [psi+])

Medium	[*psi*⁻] mutant	Total colonies scored	Phenotypes of colonies: Red	White	Red/pink/ white	Red/pink	% White colonies
YC	507 Me-1 [*psi*⁻]	352	243	63	46	0	17.9
	507 Me-3 [*psi*⁻]	255	215	37	3	0	14.5
	468/1d	534	366	109	59	0	20.4
	507 *pnm*-1	770	9	28	0	733	3.6
	507 *pnm*-2	691	3	3	0	685	0.4
Omission medium less leucine	507 Me-1 [*psi*⁻]	266	0	161	105	0	60.5
	507 Me-3 [*psi*⁻]	88	0	49	39	0	55.7
	507 *pnm*-1	571	10	25	0	536	4.4
	507 *pnm*-2	808	7	1	0	806	0.1

Phenotypes of colonies growing on OM—leu were determined after replica-plating to YC.

inherited nuclei from the *pnm* parent. It was shown that the red clones growing on YC were of two kinds: α *ura⁻ LEU⁺* (from the *pnm* parent) and **a** *URA⁺ leu⁻* (from the *kar* parent). Second, a small portion of white clones grew. These were 1/5 to 1/50 the frequency of white clones from the [*psi⁺*] × *kar* matings; 46 of them were tested and found to be *URA⁺ LEU⁺* and to sporulate well. They were, therefore, all diploids. Since no α *ura⁻ LEU⁺* clones segregated from these zygotes, we concluded that the *pnm* mutations were located in the nucleus and prevented the inheritance, maintenance or expression of the [*psi⁺*] factor from the *kar* parents.

We have found that the properties of the *kar* strain, MT 152/3c (described here) can be exploited in the form of a simple cross-streak complementation test. We have shown that, if the mutation giving rise to a nonsuppressed phenotype is nuclear, only diploids formed in these matings can be suppressed and are white. Since these are rarely formed (see Tables 3 and 5), such mutants will show only weak complementation, if any, in cross-streaks. In contrast, [*psi⁻*] mutants segregate many suppressed haploids and strong complementation will occur. Sixteen *pnm* mutations have been identified by tetrad analysis (Table 1B, lines 37–41; other data not shown). Similar tetrad analysis has confirmed the genotype of 15 [*psi⁻*] *strains*. These were 507 Me1 and 507 Me3 (Table 1); 468/1d and 483/2d (data not shown) and 11 [*psi⁻*] revertants induced in MT182/8d (α, *SUQ5* [*psi⁺*]) by treatment with guanidine hydrochloride (data to be published). These 31 strains were cross-streaked with the *kar1* tester MT152/3c on YC plates. All 15 [*psi⁻*] mutants gave a strong complementation reaction, showing vigorous white growth. All 16 *pnm* mutants showed little if any complementation. The distinction between the cytoplasmic [*psi⁻*] and the nuclear *pnm* mutations could be made even clearer when the cross-streaks were replicated, after zygotes had formed, onto YNB + uracil medium. This prevents growth of both the *leu1 kar1* tester strain and the unsuppressed strains. Haploid

segregants from *kar1 leu1* [*psi*$^+$] × *SUQ5* pnm crosses cannot grow on this medium, but those from *kar1 leu1* [*psi*$^+$] × *SUQ5* [*psi*$^-$] crosses can grow if they contain the nucleus from the latter parent because they acquire the [*psi*$^+$] factor. This form of the test was tried using four *pnm* strains and 17 [*psi*$^-$] revertants induced by guanidine hydrochloride. The results were as predicted, with all 17 [*psi*$^-$] cross-streaks growing vigorously, but only isolated colonies appearing on the replicas of *pnm* cross-streaks.

Since devising this test, we have used it on several hundred revertants of 507.4/2b and other *SUQ5* [*psi*$^+$] strains. All revertants identified as nucleur (*i.e.*, *SUQ5*$^+$ *asu ASU* or *PNM*) by the methods already described have consistently shown no complementation with the *kar* tester strain. This, together with the results already described, gives us some confidence that we can reliably distinguish nuclear from cytoplasmic mutations in this system (see Figure 2).

The spectrum of reversions

The collections of revertants of 507.4/2b, both spontaneous and induced, obtained by the methods described above were analyzed by complementation tests with the *suq5*$^+$ [*psi*$^+$] and *SUQ5* [*psi*$^-$] tester strains and with the *kar* strain MT152/3c described in the preceding section. The sets of revertants induced by mutagens and identified as red clones growing on YC medium were also tested in order to identify mutation at the *ade1* and *ade2* loci and as described in MATERIALS AND METHODS. The classification of revertants obtained is shown in Table 6.

Six of the revertants gave an unexpected complementation pattern. They all complemented the *suq5*$^+$ [*psi*$^+$] *kar1* tester strain, indicating that they had a functional *SUQ5* suppressor gene, no other nuclear mutation blocking suppression and had become [*psi*$^-$]. Consequently, and as expected, they failed to complement the *SUQ5* [*psi*$^-$] tester. However, they also failed to complement the *suq5*$^+$ [*psi*$^+$] tester, as other [*psi*$^-$] revertants do. Our interpretation of the complementation pattern is that a nuclear mutation has occurred (concomitantly with the [*psi*$^-$] mutation) that renders *SUQ5* recessive. Other workers have reported that in some genetic backgrounds, *SUQ5* becomes recessive in suppressing *ade2-1* (F. SHERMAN, personal communication) and we will describe elsewhere a genetic control of the dominance of the *SUQ5* suppressor mutation. These six revertants have not been analyzed further.

The spectra of revertants obtained by two methods of selecting spontaneous reversions (Table 6, lines 1 and 2) are similar. This suggests that neither system of selection introduces any gross distortion of the underlying spectrum of spontaneous mutation.

The spectra of induced reversions were somewhat different from the spontaneous spectrum. The most noticeable differences lay in a smaller proportion of [*psi*$^-$] mutations among the induced set and a greater proportions of *asu* muta-

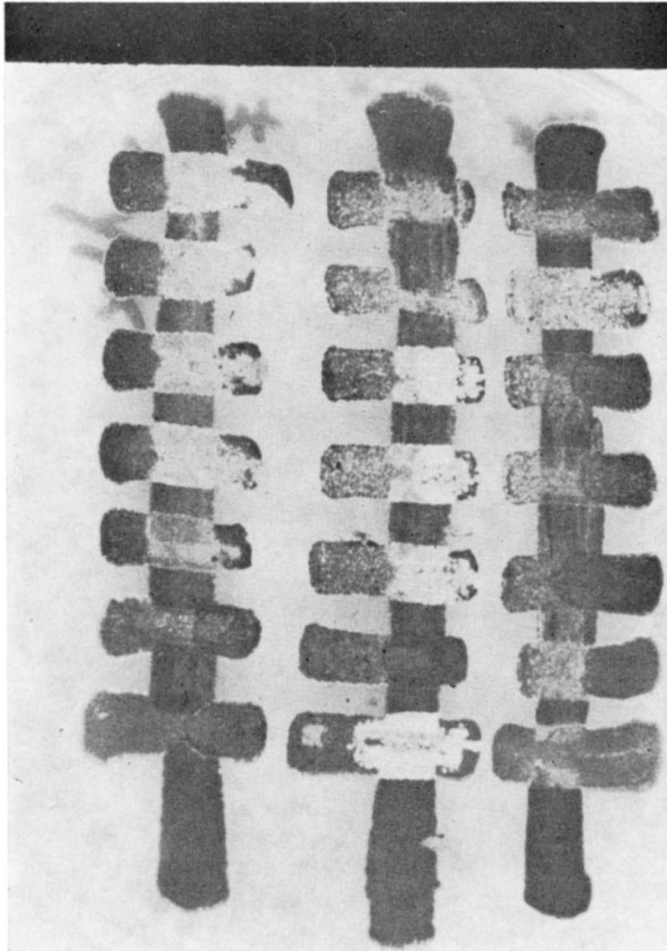

FIGURE 2.—An illustration of complementation tests to distinguish nonsuppressed revertants. The vertical streaks, from left to right, have the genotypes: **a**, *suq5*+ [*psi*+]; **a**/**a** *SUQ5*/*SUQ5* [*psi*−] *and* **a** *suq5*+ *Kar1-1* [*psi*+]. The strain streaked horizontally are revertants of 507.4/2b and, from top to bottom, were classified as follows: *pnm*, (+,—,—); [*psi*−], (+,—,+); *asu*, (+,+,—); *ade1* (+,+,—); *ade2.x* (+,+,—); *PNM* (—,—,—); and *SUQ5*+ (—,+,—). Only the cytoplasmic mutant (second from the top) complements the *Kar* tester. The complementation of the *ade2-x* revertant with the *suq5*+ [*psi*+] tester is weak because of negative complementation at the *ade2* locus. The use of a diploid for the [*psi*−] tester reduces the frequency of background reversion.

tions. The complementation tests were carried out by different operations and different methods (see MATERIALS AND METHODS), but the classifications have been cross-checked with no discrepancies. The differences are due either to artifacts introduced by the methods of screening or to peculiarities of the mutagen treatments. We have not combined mutagenesis with selection in any large-scale study.

TABLE 6

The number of nonsuppressed revertants of various classes isolated by different means from 507.4/2b = α SUQ5 [psi+] ade2-1 his5-2 lys1-1 can1-100 ura3-1

Method of isolation (see text)	Total	Non-maters	[*psi*⁻]	*SUP*+ or *Asu*	*PNM* or *ASU*	*pnm*	*asu*	Recessive *SUQ5*	*ade1*	*ade2*
Random red *can*^{R}	89	—	32	13	26	18	0	0	—	—
Random red cold resistant (YEPD at 12°)	81	—	26	16	13	19	1	6	—	—
25 µg/ml NTG										
(a) at 28°	41[1]	0	3	12	11	2	9	0	1	4
(b) at 23°	90[2]	3	17	10	3	17	20	0	6	14
80 J.m^{-2} 254 nm UV	70[3]	0	10	17	4	12	17	0	5	5

Frequencies: (1) 21.4×10^{-3} revertants at 3.8% survival.
(2) 27.0×10^{-3} revertants at 7.0% survival.
(3) 7.13×10^{-3} revertants at 3.99% survival.

DISCUSSION

We have established, by an analysis of their inheritance in tetrads, the occurrence of a number of types of mutations that affect nonsense suppression by the ochre suppressor *SUQ5* in *Saccharomyces cerevisiae*. Some of these we expected to find or have described before (Cox 1965; Young and Cox 1971; McCready and Cox 1973). Others were unexpected. The reversions were of three main categories. First, there were reversions of the suppressor, *SUQ5*, itself. Some of these may have been second-site mutations within the locus (Rothstein 1977). Second, there were mutations that affected the activity or expression of the suppressor. These include mutations of the cytoplasmically inherited [*psi*+] factor to [*psi*⁻] and various antisuppressor mutations. Third, there were nuclear mutations that affected the expression and inheritance of the [*psi*+] factor.

Although tetrad analysis is necessary to distinguish all the classes of mutation from one another, a limited classification can be achieved by the phenotypes of diploids formed by mating revertants with two strains, one *suq5*+ [*psi*+] and the other *SUQ5* [*psi*⁻]. These "complementation tests" leave three ambiguous groups. One of these ambiguities has potentially serious consequences for studies of the kinetics of induction of the cytoplasmic [*psi*⁻] mutation or patterns of inheritance of the [*psi*] factor. The novel recessive "*pnm*⁻" mutations are nuclear in origin, have the property of converting a [*psi*+] cytoplasmic genome to [*psi*⁻] and, consequently, are indistinguishable from true cytoplasmic [*psi*⁻] mutations by these tests alone. This source of embarrassment was fortunately averted by the genius of Fink and Conde (1977) in isolating the *kar* mutation. When a strain of the genotype **a**, *ade2-1*, *kar1* [*psi*+] was constructed, we found that it could be reliably used to distinguish pure cytoplasmic [*psi*⁻] mutations in general from all nuclear mutations and in particular from the *pnm*⁻ mutations. Recessive *pnm*⁻ mutations are analagous to certain *pet* and *mak* mutations required for the maintenance of [*rho*] or of the killer plasmid in yeast

(WICKNER and LEIBOWITZ 1976; LIEBOWITZ and WICKNER 1978). Fortunately, since the frequency of *[rho⁻]* mutation is so high, no ambiguity is likely to arise in mutagenesis studies of mitochondrial petites from the induction of *pet⁻* mutations. On the other hand, in studies of killer maintenance, the assay of cytoplasmic sensitives could easily be confused by numbers of *mak⁻* mutations. It is clear that the "*kar*" test could be applied in a similar way to distinguish *MAK+* [*kil⁰*] from [*mak⁻*] mutants.

The other two ambiguities inherent in these cross-streak tests of nonsuppressed revertants are unimportant for studies of [*psi*] mutagenesis, and we have not attempted to design tests to resolve them. They are interesting in that they follow from the occurrence of two unexpected kinds of antisuppressors. One of these is wholly dominant (*ASU⁻*) and, in the complementation tests, behaves similarly to the dominant *PNM⁻* mutations. In principle, a fourth tester strain of genotype *SUQ5 kar1* [*psi⁻*] would discriminate between them, since *ASU⁻* is still [*psi+*] and would allow suppressed *SUQ5 kar1* [*psi+*] cells to segregate from the cross. The other antisuppressor, *Asu⁻*, is one that is effective in *SUQ5/+* heterozygotes but not in *SUQ5/SUQ5* homozygotes unless it itself is homozygous. That is, of the set of [*psi+*] strains: *SUQ5 Asu*, *SUQ5/SUQ5 Asu/Asu*, *SUQ5/+ Asu/+* and *SUQ5/SUQ5 Asu/+*, only the last is suppressed. The last two are the diploids formed by crossing an *Asu* revertant with the *suq5+* [*psi+*] and *SUQ5* [*psi⁻*] testers, respectively; hence, this revertant is indistinguishable from a *sup5+* revertant, by these tests.

Previously, only recessive antisuppressors (*asu⁻*) have been found. Leaving aside regulatory mutants, the occurrence of semidominant *Asu⁻* and dominant *ASU⁻* mutations suggests some interesting controls of suppressor activity. Recessive antisuppressors may arise through a variety of defects in the translation mechanism (MCCREADY and COX 1973), including mutant *tRNA* modification or maturation enzymes, deficient amino-acyl *tRNA* synthetases, and mutant ribosomal proteins or *rRNA* sequences. Dominant antisuppressors cannot be the consequence of mutations that merely delete a function from the cell. They could, however, arise through mutations producing an altered *tRNA* methylase that inactivates suppressor *tRNA* by erroneous or misplaced methylations. A gene producing a nuclease that acts on an ochre anticodon sequence would also be dominant, as would a "splicing enzyme" that made a mistake specifically in suppressor *tRNA*. A fifteen-base insert in the *tyr-tRNA* genes of yeast lies adjacent to the anticodon (GOODMAN, OLSON and HALL 1977). The most likely reason for semidominance is an alteration of read-through levels such that in half-dose (*SUQ5/+*) insufficient suppressor *tRNA* is present to affect the phenotype. This could be caused by altered termination factors or ribosomal proteins. Other possibilities are "leaky" versions of dominant antisuppressors, or activating or modifying enzymes whose affinity for suppressor *tRNA* is so lowered that, in the presence of the wild-type *tRNA*, competition ensures that no active suppressor *tRNA* is produced. Both dominant and semidominant antisuppressors, then, suggest the occurrence of enzymes able to discriminate between suppressor and nonmutant *tRNA*.

Dominant PNM^- and recessive pnm^- mutants also demand different kinds of explanations. Recessive mutations are most simply explained as the loss of enzymatic function necessary for the maintenance of [*psi*$^+$]. Not all are so simple because, although masked in the heterozygous diploid, some are dominant or partly dominant during sporulation since the tetrads segregate an excess of [*psi*$^-$] spores (Table 1). The truly dominant PNM^- not only reflects this but shows no sign of [*psi*$^+$] activity in *PNM/+* diploids. These are likely to be either regulatory mutants producing a "super-repressor" or they may specify enzyme activities that destroy [*psi*$^+$] determinants. We shall describe the range of properties of nuclear genes controlling the [*psi*] factor in more detail elsewhere.

Finally, this study has shown that the [*psi*$^+$] to [*psi*$^-$] mutation occurs with a frequency comparable to that of forward mutations in nuclear genes, both spontaneously and when induced by powerful nuclear mutagens. In this, it differs from the cytoplasmic petite mutation. The frequency of [*psi*$^-$] revertants may reflect a segregational probability. However, many [*psi*$^-$] mutants appear to revert to [*psi*$^+$] at a comparable frequency (unpublished results) and these, therefore, cannot have arisen by loss of a determinant through its failure to segregate. The nature of [*psi*$^-$] mutations has yet to be resolved.

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Corresponding editor: R. E. ESPOSITO