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Allosuppressors in yeast

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SUMMARY

Five loci have been identified in *Saccharomyces cerevisiae* whose function reduces suppressor activity in strains carrying ochre super-suppressor mutations. Recessive mutations which allow an increased level of suppression occur at these loci. In such mutants, termed allosuppressors, the serine-inserting suppressor *SUPQ5* suppresses ochre mutations in a [*psi*$^-$] background and Class I tyrosine-inserting suppressors are lethal or have a reduced viability. Mutations at two allosuppressor loci, *sal3* and *sal4*, have a lethal interaction with one another and with the extrachromosomal determinant [*psi*$^+$]. This interaction is expressed in the absence of any suppressor mutation. All the mutant alleles of one allosuppressor locus *sal3* are cold sensitive. One allosuppressor mutation, *sal4.2*, is temperature-sensitive for growth, as well as for other aspects of its phenotypic expression; namely the expression of *SUPQ5* and the lethal interactions with Class I super-suppressors, with [*psi*$^+$] and with *sal3*. At low temperature (24 °C), *sal3-sal4.2* double mutants weakly suppress the ochre mutation *ade2.1*, but do not suppress *his5.2* or *lys1.1*. It is argued that the site of function of the products of these loci is ribosomal and that they are involved in chain termination at UAA codons. It is inferred that the [*psi*$^+$] factor or its product affects protein synthesis by interaction with the ribosome.

1. INTRODUCTION

In the yeast *Saccharomyces cerevisiae*, a super-suppressor, *SUPQ5*, has been described which suppresses ochre mutations, inserting serine at the point of this chain-terminating triplet (Liebman, Stewart & Sherman, 1975). An amber-suppressing revertant of SUPQ5 has been shown to contain a novel species of serine-accepting tRNA (Capecchi, Hughes & Wahl, 1975). The efficiency of the activity of the ochre suppression is governed by an extra-chromosomal genetic determinant named [*psi*], so that in [*psi*$^+$] strains, all ochre mutations so far assayed are suppressed, whereas in [*psi*$^-$] strains, many are not suppressed at all and others suppressed only very weakly and sporadically (Cox, 1965, 1971; Liebman *et al.* 1975). Thus a strain of genotype *ade2.1*, *SUPQ5*, [*psi*$^+$] is prototrophic while one of genotype *ade2.1*, *SUPQ5*, [*psi*$^-$] requires adenine for growth. A diploid formed by mating two such strains is prototrophic, as are all the spore cultures derived from it.

We have shown that an adenine requiring strain of the genotype *ade2.1*, *SUPQ5*,

[*psi*$^-$] reverts at a high frequency to prototrophy ($2{\cdot}5 \times 10^{-6}$) (Young & Cox, 1975; Cox, Sieuw Keen Quah & von Borstel, unpublished). This mutation frequency is about 100 times the frequency of reversion in adenine requirers of genotypes *ade2.1*, *supQ5*$^+$, [*psi*$^+$] or *ade2.1*, *supQ5*$^+$ [*psi*$^-$] ($1{\cdot}4 \times 10^{-8}$). Reversions are of three kinds. These are:

(1) Reversions from [*psi*$^-$] to [*psi*$^+$].

(2) Dominant suppressor mutations which may or may not segregate independently of *SUPQ5* and, like many other super-suppressors, do not depend on [*psi*$^+$] for expression of the suppressed phenotype.

(3) Chromosomal mutations that have the effect of allowing the expression of *SUPQ5* as a suppressor while the strain remains [*psi*$^-$]. These mutations by themselves do not suppress either ochre or amber mutations. They are recessive (Young & Cox, 1975).

About 90 % of the revertants obtained are of the third kind. It is clearly this class of mutations which accounts for the remarkable instability of the auxotrophic parent strains. Following the precedent of Hawthorne (Hawthorne, 1967), these mutations will be called 'allosuppressors' (*sal*).

The high rate of spontaneous mutations of this kind suggests that a relatively large number of loci may mutate to give this phenotype: possibly ten times the number which can mutate to produce Class I suppressors (Schuller & von Borstel, 1974). If the allosuppressor mutations are additional suppressor tRNAs, these loci would be expected, like the determinants of Class I suppressors, to be genetically unlinked; scattered, on the whole, throughout the yeast genome (Hawthorne & Mortimer, 1968). Alternatively, there may be only a few genes each with several sites which may mutate to give this phenotype. Either possibility suggests an interesting class of mutations affecting the fidelity of translation of messenger RNA. This paper reports a genetic analysis of allosuppressor mutations.

2. MATERIALS AND METHODS

(i) *Strains*. All the strains used are described at the appropriate point in the 'Results' section. All are ultimately derived from crosses involving strains described in an earlier paper (Cox, 1965), notably segregants of diploids 193 (*ade2.1*/*ade2.1*, *SUPQ5*/*SUPQ5* [*psi*$^-$] and 406 (*ade2.1*/*ade2.1*, *SUPQ5*/*SUPQ5*, [*psi*$^+$]) with poly-auxotrophic revertants of X2315-14C (α, *ade2.1*, *his5.2*, *lys1.1*, *trp5.48*, *can1.100*, *ura3.1*, *SUP3.1*). This was the source of the *SUP3.1* strain used in crosses described in the text. The *SUP2.1* strain used was X2314-8C (*ade2.1*, *his5.2*, *lys1.1*, *trp5.48*, *can1.100*, *ura3.1*, *SUP2.1*). X2315-14C and X2314-8C were provided by the Yeast Stock Center, University of California, Berkeley, U.S.A. The *SUP11.2* strain was described in McCready & Cox, 1973.

(ii) *Media*. YC (yeast complete) is a rich growth medium described by Cox & Bevan (1962).

YNB is Difco Yeast Nitrogen Base without amino-acids, as described in the Difco manual. It is a minimal medium and was suitably supplemented so as to

support the growth of auxotrophic strains or to score auxotrophic requirements or resistance to canavanine. Amino-acids, adenine and uracil were added, when necessary, at 10 μg/ml final concentration and canavanine at 30 μg/ml.

Sporulation medium was that described by Hurst & Fogel (1964).

(iii) *Methods.* Unless otherwise described, all diploids intended for phenotypic and genetic analysis were isolated by picking single zygotes with the aid of a micromanipulator from cells mixed together 4–6 h earlier on YC agar (Cox & Bevan, 1962). Tetrad analysis has been described (Johnston & Mortimer, 1959).

Many of the genes to be described in this study do not have an immediately identifiable phenotype, showing their presence only through their interaction with other genes. For example, mutant and non-mutant alleles of *SUPQ5* cannot be distinguished in [*psi*$^-$] strains. The suppressible ochre mutation *ade2.1* was present in all strains. When this gene is non-functional, cultures accumulate a red pigment. Suppressed strains, like wild type, are white in colour. This colour marker is a reliable indicator of suppressor activity when cultures are grown on YC medium, even when the strains are petite. It is less reliable when the more conventional 1 % yeast-extract, 1 % peptone, 2 % dextrose medium (YEPD) is used. However, YEPD gives identical results to YC if the yeast extract is reduced to 0·5 % and the dextrose increased to 4 %.

With the *ade2.1* mutation present, the presence or absence of the various components of the suppression system can often be determined by phenotypes of diploids formed by appropriate crosses. When the two haploids to be crossed were of the same colour, the cross-streak method described by McCready & Cox (1973) was generally used. When they were of different colours, three or four single zygotes were isolated from a mating mixture and grown for three days or more into sizeable colonies. The principal examples encountered in the text are as shown below.

Text page	Genotypes to be distinguished	Tester strains	Diploid phenotype × *sal1*	Diploid phenotype × *sal2*
8	*sal1* +	*SUPQ5 sal1*	W	R
	+ *sal2*	and	R	W
	sal1 sal2	*SUPQ5 sal2*	W	W
10	*SUPQ5 psi*$^-$ / *supQ5*$^+$ *psi*$^-$	*supQ5*$^+$ *psi*$^+$	W / R	
10, 12	*SUP3* − − / + *SUPQ5 sal4*	*SUPQ5 sal3*	W / R	
14	*sal4 psi*$^+$ / + *psi*$^+$	*SUPQ5 sal4 PNM*	W / R	
17	*PNM* / *pnm*$^+$	*SUPQ5 psi*$^+$	R / W	

Some genotypes, for example *SUP2 SUPQ5* and *SUP2 supQ5*$^+$, *SUP2 sal* an *SUP2 SAL*$^+$, cannot be distinguished without further tetrad analysis.

3. RESULTS

(1) *Provenance*

Two strains were used to gather allosuppressor mutations. One, 502/2a, of genotype *a*, *ade2.1*, *his5.2*, *lys1.1 can1.100*, *SUPQ5*, [*psi*$^-$], was derived from a line descended from one of the [*psi*$^-$] mutants described in 1965 (Cox, 1965). The other, 503/4b, of genotype *α*, *ade2.1*, *lys1.1*, *SUPQ5*, [*psi*$^-$], was a segregant from a cross in which one of the parents was [*psi*$^-$] and the other carried the *PNM2.19* mutation. *PNM* mutations, originally called *R*, behave as if they eliminate [*psi*$^+$] determinants from strains carrying them (Young & Cox, 1971; McCready, Cox & McLaughlin, 1977).

Table 1. *The numbers of replicas of colonies examined and the numbers of papillae observed*

Plate no.	No. of colonies examined	Papillae on unirradiated replicas	Papillae on irradiated replicas	UV dose (J.m^{-2})
		(*a*) Strain 502/2a		
1	87	14	79	5
2	38	7	104	10
3	48	5	295	15
4	134	4	375	20
5	181	10	868	25
6	82	12	440	30
7	98	7	478	35
8	77	16	160	40
		(*b*) Strain 503/4b		
11	131	24	140	5
12	153	10	372	10
13	134	9	633	15
14	114	13	609	20
15	172	16	501	25
16	129	11	486	30
17	106	13	289	35
18	107	21	184	40
19	142	8	288	45
20	108	15	138	50

Each strain was plated on a set of YC plates so as to yield about 200 colonies on each plate. The YC plates were then replicated each to two YNB plates. One set of replicas was irradiated with various doses of ultraviolet light (254 nm wavelength), leaving its companion set unirradiated. After incubation for five days, the plates were examined. Prototrophic papillae had formed on many of the colony replicas and the numbers were recorded. On 745 unirradiated replicas of colonies of strains 502/2a, 76 papillae were counted (0·10 papillae/colony) and on 1296 unirradiated colonies of 503/4b, there were 140 papillae (0·11/colony). The identical colony replicas, irradiated, gave much larger numbers (Table 1). For example, on one plate, 114 replicas of colonies of 503/4b, unirradiated, yielded 13 papillae. The

same 114 replicas on a second plate yielded 609 papillae after irradiation with 20 $J.m^{-2}$ light at 254 nm. These numbers cannot be converted into mutation frequencies because no accurate estimate is possible of the number of cells transferred by replica plating to the YNB plates, nor of their survival after UV-irradiation. However, they illustrate the ready UV-inducibility of this type of reversion.

Individual papillae were picked from these sets of colony replicas, no more than one papilla from each colony, yielding for further analysis about 170 spontaneous revertants of independent origin and about 200 induced by UV. The picked papillae were re-purified by streaking inocula on YNB plates.

(ii) *Classification*

(*a*) [Psi$^+$] *Reversions*

The prototrophic revertants were cross-streaked with strains of genotype: *a* or α, *ade2.1*, *his5.2*, *lys1.1*, *can1.100*, *ura3.1*, *SUPQ5*, [*psi*$^-$]. After 24 h incubation to allow diploids to form, the cross-streaks were replica-plated to YNB supplemented with adenine, histidine, lysine and canavanine and to sporulation medium. The latter replicas were incubated to allow asci to form and then replicated to YNB with the same supplements. Any revertants which are [*psi*$^+$] form diploids which are homozygous for the suppressor (*SUPQ5/SUPQ5*) and [*psi*$^+$]. Not only are such diploids themselves canavanine sensitive, but in them, no canavanine-resistant spores segregate at meiosis and no growth is recorded on the supplemented YNB plates. All other classes of revertants segregate canavanine-resistant spores which are also adenine-requiring and grow as red cultures.

Among 58 spontaneous and 100 UV-induced revertants of 502/2a and 108 spontaneous and 106 UV-induced revertants of 503/4b, no [*psi*$^+$] mutations were identified.

(*b*) *Complementation analysis*

Allosuppressor mutations are recessive. Thus a diploid of genotype

$$\frac{a\ ade2.1}{\alpha\ ade2.1}\ \frac{SUPQ5}{SUPQ5}\ \frac{sal}{+}\ [psi^-]$$

is red and adenine-requiring. This provides a simple test for allelism of allosuppressor mutations. Revertants of opposite mating-type are cross-streaked on YC and the distal portions of the streaks examined later for red diploid growth.

Initially, ten spontaneous revertants of 502/2a and fifteen of 503/4b were crossed with each other and with four allosuppressor mutations isolated in an earlier study which were available in both mating-types. These 29 mutants fell into a consistent complementation pattern, part of which is shown in Table 2. The mutants unrecorded in this table failed to complement any other mutant and were scored, for the time being, as dominant suppressor mutations. The Table identifies at least three complementing loci. The α-mating-type mutants 532-3 (designated *sal1*), 503/4b-W11 (*sal2*) and 503/4b-W3, -W6 and -W8 (representing up to three addi-

tional loci) were used as testers in crosses with the remaining 19 spontaneous revertants of 502/2a. These showed that the latter three testers (503/4b-W3, -W6 and -W8) all belonged to a single complementation group, now designated *sal3*. A fourth complementation group, represented by mutant 502/2a-W24, was identified and designated *sal4*. It was possible to assign all but three of the 502/2a

Table 2. *The complementation pattern of 13 allosuppressor mutations*

	532-3a	532-2a	533-3a	533-7a	502-W3	502-W5	502-W8	502-W10
532-3α	0	0	0	0	0	0	+	+
532-2α	0	0	0	0	0	0	+	+
533-3α	0	0	0	0	0	0	+	+
533-7α	0	0	0	0	0	0	+	+
503-W3α	+	+	+	+	+	+	+	+
503-W6α	+	+	+	+	+	+	+	+
503-W8α	+	+	+	+	+	+	+	+
503-W11α	+	+	+	+	+	+	0	0
503-W14α	0	0	0	0	0	0	+	+

Table 3. *Complementation tests of white revertants of 502/2a*

532-3 (*sal1*)	503-W11 (*sal2*)	503-W3 (*sal3*)	503-W6 (*sal3*)	503-W8 (*sal3*)	Revertant no.	
0	0	0	0	0	502-W11	Non-complementing
0	+	+	+	+	12	*sal1*
0	0	0	0	0	13	Non-complementing
+	+	0	0	0	14	*sal3*
0	+	+	+	+	15	*sal1*
+	0	+	+	+	16	*sal2*
+	+	0	0	0	19	*sal3*
0	+	+	+	+	21	*sal1*
+	0	+	+	+	22	*sal2*
+	+	+	+	+	24	*sal4*
+	+	0	0	0	25	*sal3*
0	+	+	+	+	27	*sal1*
+	0	+	+	+	29	*sal2*
+	+	0	0	0	32	*sal3*
0	0	0	0	0	33	Non-complementing
0	+	+	+	+	34	*sal1*
+	+	0	0	0	35	*sal3*
0	+	+	+	+	37	*sal1*
0	+	+	+	+	38	*sal1*

spontaneous revertants to one of the four complementation groups (Table 3). The 502/2a revertants are *a* mating-type. From them, a single representative of each complementation group was used to classify 58 spontaneous and 44 UV-induced revertants of 503/4b, which are α mating-type. Finally, 46 UV-induced revertants of 502/2a were classified. In these final steps, four mutants were found belonging to a fifth complementation group (*sal5*). All mutants could be classified unambiguously in the sense that each one complemented mutants in four of the five groups or else failed to 'complement' any of them and was classified as a dominant

Table 4. *A summary of tests of allelism by complementation among revertants of 502/2a, 503/4b and other strains*

(The figures are the number of mutants in each category.)

Complementation group	'Dominant'	*sal1*	*sal2*	*sal3*	*sal4*	*sal5*
Revertants from 502/2a						
(*a*) Spontaneous	5	10	5	6	1	0
(*b*) UV-induced	0	8	6	18	11	3
Revertants from 503/4b						
(*a*) Spontaneous	24	9	7	17	6	1
(*b*) UV-induced	2	0	0	37	5	0
Others (533, 532, 553/25a; all spontaneous)	0	11	3	8	1	3
Totals	31	38	21	86	24	7

Table 5. *Recombination between members of the five allosuppressor complementation groups*

(The numbers in each box are of parental (P), non-parental (N) and tetratype (T) tetrads recovered, displayed thus $\frac{P \quad N}{T}$.)

	sal2	*sal3*	*sal4*	*sal5*
sal1	*sal1.1* × *sal2.10* $\frac{2 \quad 5}{1}$	*sal1.1* × *sal3.5* $\frac{0 \quad 0}{9}$	*sal1.1* × *sal4.2* $\frac{2 \quad 0}{8}$	*sal1.1* × *sal5.3* $\frac{1 \quad 1}{8}$
sal2	.	*sal2.1* × *sal3.5* $\frac{0 \quad 4}{6}$	*sal2.1* × *sal4.2* $\frac{1 \quad 2}{5}$	*sal2.1* × *sal5.3* $\frac{6 \quad 3}{8}$
sal3	.	.	*sal3.5* × *sal4.1* $\frac{1 \quad 1}{7}$	*sal3.5* × *sal5.3* $\frac{4 \quad 7}{14}$
			sal3.5 × *sal4.2* $\frac{2 \quad 0}{8}$	*sal3.2* × *sal5.3* $\frac{6 \quad 1}{3}$
sal4	.	.	.	*sal4.2* × *sal5.3* $\frac{2 \quad 1}{7}$

mutation. The results are summarized in Table 4. A handful of mutants were not classified because they grew rather poorly.

(*c*) *Intragenic complementation*

A limited study of intragenic complementation was made amonst *sal1* and *sal3* isolates. In the *sal1* group, 13 spontaneous isolates of *a* mating-type were crossed with 13 α isolates. No complementation was observed. Similarly, four *sal3*, *a* isolates were crossed with 22 *sal3*, α strains and again, no complementation was observed.

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(iii) *Recombination*

(*a*) *Intergenic recombination*

A few tetrads from crosses of representatives of each of the five complementation groups were analysed. The segregation of the allosuppressors was followed by complementation tests. (See 'Materials and Methods'.) All allosuppressor mutations segregated 2 : 2 in tetrads. There is no evidence of linkage between the complementation groups (Table 5). A lethal interaction was observed between alleles of *sal3* and *sal4*. When the temperature-sensitive mutation, *sal4.2* was involved in a cross with a *sal3* allele (*sal3.5*), this lethality was expressed at 28 °C, but not at 24 °C.

At 24 °C, this double mutant (*sal3.5*, *sal4.2*) suppressed *ade2.1* very weakly, but did not suppress *his5.2* or *lys1.1*. All these interactions were independent of the presence of *SUPQ5*.

There was also evidence of reduced viability of *sal2.1*, *sal4.2* double mutants.

(*b*) *Intragenic recombination*

Diploids were made by crossing the following pairs of alleles within each complementation group: *sal1.1* × *sal1.3*; *sal2.1* × *sal2.10*; *sal3.1* × *sal3.5*; *sal4.1* × *sal4.2* and *sal5.1* × *sal5.3*. All diploids were also homozygous for *SUPQ5*. Twenty tetrads were dissected from each. The following numbers of spores germinated: *sal1.1* × *sal1.3*; 73; *sal2.1* × *sal2.10*: 79; *sal3.1* × *sal3.5*: 74; *sal4.1* × *sal4.2*: 79, and *sal5.1* × *sal5.3*: 78. No non-suppressed recombinants were recovered.

The conclusions to be drawn from the tests of complementation and recombination are that there are five chromosomally located loci in yeast at which recessive mutations may occur which permit *SUPQ5* to be expressed in a [*psi*$^-$] background. An approach to understanding the function of these loci may be made by considering their interactions with other mutant loci.

(iv) *Interactions of allosuppressors*

(*a*) *Interactions with Class I suppressors*

Allosuppressors appear to be chromosomal mimics of the extra-chromosomal [*psi*$^+$]-factor in that by themselves they show no evidence of suppressor activity, but when the *SUPQ5* suppressor mutation is present, suppression is observed. The [*psi*$^+$]-factor has been shown to act by increasing the efficiency with which *SUPQ5* acts (Liebmann *et al.* 1975). It also increases the efficiency of suppression by other ochre suppressors, notably the Class I, tyrosine-inserting suppressors. This is manifested by the observation that in a [*psi*$^+$] background, these suppressors are lethal. It is suggested that in such strains, the efficiency of read-through of ochre codons by suppressor tRNAs is increased to such an extent that normal protein synthesis is severely affected, and the cells die (Cox, 1971).

Alleles of the various allosuppressor loci were crossed with strains carrying other ochre suppressors and tetrads analysed to determine whether they similarly affected the efficiency of other suppressor mutations. The segregation of a few

selected tetrads from one such cross is illustrated in Table 6 and a summary of the remaining tetrad analyses in Table 7.

Table 6 illustrates some of the problems associated with analysing the segregants from these crosses. First, it will be noted that in some tetrads one or two spores failed to germinate. Secondly, not all the segregants have been scored for all three heterozygous markers. This is because some genotypes cannot be determined

Table 6. *The segregation of* SUPQ5, SUP11.2 *and* sal3.5 *in five tetrads from a diploid heterozygous for all three markers and homozygous* ade2.1/ade2/1

Tetrad and spore number	Ability to grow without adenine	Distribution of markers		
		SUP11.2	*SUPQ5*	*sal3.5*
1a	−	+	+	.
1b	−	+	+	.
1c, 1d	Did not germinate			
2a	−	+	*SUPQ5*	+
2b	−	+	+	.
2c	+	*SUP11.2*	.	.
2d	Did not germinate			
3a	−	+	*SUPQ5*	+
3b	+	*SUP11.2*	.	.
3c	+	+	*SUPQ5*	*sal3.5*
3d	Did not germinate			
7a	+	*SUP11.2*	.	.
7b	+	+	*SUPQ5*	*sal3.5*
7c	+	*SUP11.2*	.	.
7d	−	+	+	.
47a	+	*SUP11.2*	.	.
47b	+	+	*SUPQ5*	*sal3.5*
47c	+	+	*SUPQ5*	*sal3.5*
47d	+	*SUP11.2*	.	.

Table 7. *The numbers of tetrads in which 4, 3, 2, 1 or no spores germinated when incubated after dissection from diploids heterozygous for both an allosuppressor and a Class I suppressor*

(Numbers in parentheses indicate the number of cultures which were likely to be double mutants of the type *SUP-sal.*)

Class I suppressor ...	*SUP2.1*						*SUP3.1*						*SUP11.2*					
No. of spores which germinated ...	4	3	2	1	0		4	3	2	1	0		4	3	2	1	0	
Allosuppressor																		
1·1	.	.	.	.	.		4	6	0	0	0	(1)	5	2	2	1	0	(3)
2·1	.	.	.	.	.		6	4	0	0	0		.	.	.	.	.	
3·1	.	.	.	.	.		1	4	5	0	0		.	.	.	.	.	
3·3	.	.	.	.	.		.	.	.	.	.		2	2	6	0	0	
3·5	4	28	8	0	0		2	4	3	0	1		10	52	14	1	1	
3·7	.	.	.	.	.		.	.	.	.	.		1	5	4	0	0	
4·1	.	.	.	.	.		2	3	5	0	0	(1)	.	.	.	.	.	
4·2 (24 °C)	.	.	.	.	.		5	5	0	0	0	(8)	.	.	.	.	.	
(28 °C)	17	2	0	0	0	(13)	3	7	0	0	0		.	.	.	.	.	

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without crossing the spore cultures and performing further tetrad analysis. For example, in the presence of *SUP11.2*, the presence or absence of *SUPQ5* cannot be determined, since the strain is suppressed anyway. Similarly, the presence of the allosuppressor cannot be ascertained in such a strain. However, an adenine-independent strain (ADE$^+$) which is so because of the combination SUPQ5–*sal3*, is distinguishable from one where the *ade2.1* mutation is suppressed by *SUP11.2* because the former complements a *sal4* (or *sal1* or *sal2* or *sal5*) strain to form a red diploid while *SUP11.2*, being dominant, does not. Similarly, adenine requiring strains which carry *SUPQ5* can be distinguished from those which do not by crossing them with a strain of genotype *supQ5*$^+$, [*psi*$^+$], when the former will yield white

Table 8. *The segregation of Class I suppressors in tetrads from diploids which were heterozygous for both the Class I suppressor and for an allosuppressor mutation*

(Only tetrads in which less than four spores germinated are shown.)

	Tetrades in which three spores germinated			Tetrads in which two spores germinated		
Diploid	*2SUP* *1sup*$^+$	*1SUP* *2sup*$^+$	*0SUP* *3sup*$^+$	*2SUP* *0sup*$^+$	*1SUP* *1sup*$^+$	*0SUP* *2sup*$^+$
SUP2 × *sal3.5*	0	14	1	0	2	1
SUP2 × *sal4.2* (24 °C)	1	3	1	0	0	0
SUP2 × *sal4.2* (28 °C)	0	7	0	0	0	0
SUP3 × *sal1.1*	1	5	0	0	1	1
SUP3 × *sal2.1*	0	3	1	0	0	0
SUP3 × *sal3.1*	0	1	1	0	7	0
SUP3 × *sal4.1*	0	3	0	0	3	2
SUP11.2 × *sal1.1*	0	2	0	1	1	1
SUP11.2 × *sal3.3*	1	1	0	0	4	2
SUP11.2 × *sal3.5*	1	25	2	0	1	13
SUP11.2 × *sal3.7*	2	3	0	0	4	0
Expected ratio	*1* :	*1*		*1* :	*4* :	*1*

suppressed diploids. Although the analysis of segregation of the markers is incomplete, the segregations observed suggest that the double-mutant combination of a Class I suppressor with an allosuppressor is lethal or of reduced viability. This is because when only three spores germinated from a tetrad, generally speaking only one of the three carried the Class I suppressor that was segregating in the diploid at meiosis. A tetratype segregation (*sal*, *SUP*; *sal*, +; +, *SUP*; +, +) yields this class when the double mutant is lethal. Had the inviability been random, of the tetrads in which only three spores germinated, half would be expected to segregate two cultures carrying the Class I suppressor, and the other half would include one culture with it. Similarly, it was found that, when only two spores had germinated in a tetrad, there was an excess of diads in which neither culture carried the Class I suppressor (the non-parental ditype segregation). These observations are illustrated in Table 8. The data show a significant departure from what is expected from random inviability when those from crosses of *SUP3.1* with various allosuppressor mutations are pooled; or when individual crosses involving

the allosuppressor *sal3.5* with different Class I suppressors are considered. A few possibly exceptional segregants could be identified. These were tetrads in which all four spores germinated, but it was clear that only one of the *sup*$^+$ segregants carried the allosuppressor mutation. For example, tetrad 534/3 was

3a = + *SUPQ5* +
3b = *SUP3*
3c = + *SUPQ5 sal1.1*
3d = *SUP3.*

If *sal1.1* segregated regularly in this tetrad, one of the two viable *SUP3* cultures must also have carried the allosuppressor. The numbers of these exceptional tetrads are recorded in parentheses in Table 7.

The inviability of the double-mutant combination is also illustrated in crosses in which *SUPQ5* was homozygous and the allosuppressor and a Class I suppressor heterozygous:

$$\frac{a}{\alpha}\ \frac{ade2.1}{ade2.1}\ \frac{SUPQ5}{SUPQ5}\ \frac{sal3.5}{+}\ \frac{+}{SUP}\ [psi^-]$$

In such diploids, a precise prediction about the tetrad types can be made:

Parental ditype	Non-parental ditype	Tetratype
SUPQ5 SUP +	*SUPQ5 SUP sal3.5**	*SUPQ5 SUP sal3.5**
SUPQ5 SUP +	*SUPQ5 SUP sal3.5**	*SUPQ5 SUP* +
SUPQ5 + *sal3.5*	*SUPQ5* + +	*SUPQ5* + *sal3.5*
SUPQ5 + *sal3.5*	*SUPQ5* + +	*SUPQ5* + +
4:0†	0:2†	2:1†

† Segregation of adenine requirement when * are inviable (+ : −).

The segregations observed from crosses involving three Class I suppressors are shown in Table 9. It is clear that the pattern of inviability is non-random and is consistent with the hypothesis that the combination of the allosuppressor with a Class I suppressor is lethal. There are two inconsistent tetrads out of 50 dissected.

One of the allosuppressor mutations isolated is a mutant conditional on temperature for growth. *sal4.2* is able to grow at 28 °C but not at 34 °C. This phenotype can be used to follow its segregation in tetrads in the absence of *SUPQ5* or the presence of dominant Class I suppressors. A diploid was formed of the genotype:

$$566 = \frac{a}{\alpha}\ \frac{ade2.1}{ade2.1}\ \frac{his5.2}{+}\ \frac{lys1.1}{+}\ \frac{trp5.48}{+}\ \frac{can1.100}{+}$$
$$\frac{ura3.1}{+}\ \frac{SUP3.1}{+}\ \frac{+}{SUPQ5}\ \frac{+}{sal4.2}\ [psi^-].$$

Tetrads from this diploid were dissected and allowed to germinate at either 24 or 28 °C. The germination patterns are recorded in Table 7. Although all the spores

dissected out germinated at 24 °C, five grew only into micro-colonies which could not be subcultured. The germination recorded is of colonies which could be subcultured. In Table 10, the genotypes of cultures in tetrads germinated at each temperature is recorded. The segregation of *sal4.2* was followed by the segregation of the inability to grow at 34 °C and of *SUP3* by the segregation of white cultures

Table 9. *The segregation of the adenine requirement in tetrads from diploids of genotype:*

$$\frac{a}{\alpha}\ \frac{ade2.1}{ade2.1}\ \frac{SUPQ5}{SUPQ5}\ \frac{SUPClassI}{+}\ \frac{+}{sal3.5}\ [psi^-]$$

(Segregations marked * are inconsistent with the hypothesis that spores of genotype *SUP* Class I – *sal3.5* are inviable. The expected numbers quoted are (*a*) those expected among 50 tetrads with a randomly distributed spore survival frequency of 0·715 (the observed frequency of survival), assuming suppressor and allosuppressor to be unlinked; not shown in the table are the numbers of tetrads expected with no spores surviving (0·33, observed = 2) or with only one (3·31, observed = 0); and (*b*) the numbers of tetrads expected if the double-mutant genotype is lethal.)

Class I suppressor in the diploid	Segregation of *ADE*+ *ade*−								
	4:0	0:2	2:1	3:1*	2:2*	3:0	1:2*	2:0	1:1
SUP2.1	2	3	12	0	0	1	0	1	1
SUP3.1	1	2	3	0	1	0	1	0	1
SUP11.2	4	1	13	0	0	1	0	0	0
Totals	7	6	28	0	1	2	1	1	2
Expected									
(*a*)	2·18	0·35	12·15	8·72	2·18	6·95	1·74	6·58	5·54
(*b*)	6·83	6·83	27·34	0	0	.	.	.	.

which failed to complement strains of genotype *a* or *α*, *ade2.1*, *SUPQ5*, *sal3*. Among those germinated at 28 °C, there are two in which *SUP3* segregated irregularly. Of the others one is a parental ditype with regard to *SUP3.1* and *sal4.2* in which all four spores germinated and seven are apparently tetratypes, in which one spore failed to germinate. In each of these, it is the double-mutant genotype, *SUP3.1*, *sal4.2*, which is missing.

Among the tetrads germinated at 24 °C, there is one in which *SUP3.1* segregated irregularly. Among the rest, there are eight segregants of the double-mutant genotype *SUP3.1*, *sal4.2*. All of these grew very poorly even at 24 °C and failed to grow at all when subcultured and incubated at 28 °C. A second diploid of similar genotype, but heterozygous for *SUP2.1* rather than *SUP3.1* was also analysed. In this case, germination was nearly normal at 28 °C, but again, all 13 *SUP2.1*, *sal4.2* double-mutants recovered grew very slowly. Two observations, then, suggest that the double-mutant genotype combining a Class I suppressor with *sal4.2* is inviable or of reduced vigour. The first is the apparent coincidence of lethality with this genotype among segregants of diploid 566 germinated at 28 °C. The second is the poor growth of such cultures when they are recovered, for example by allowing spores to germinate at 24 °C.

Table 10. *The segregation of* SUP3 *and* sal4.2 *in tetrads germinated at two different temperatures*

	(*a*) 24 °C						(*b*) 28 °C				
1a	+	+	6a	*sal4*	*SUP3*	11a	+	*SUP3*	16a	+	*SUP3*
1b	+	*SUP3*	6b	+	+	11b	*sal4*	+	16b	*sal4*	+
1c	*sal4*	*SUP3*	6c	+	+	11c	+	+	16c	*sal4*	+
1d	.	.	6d	*sal4*	*SUP3*	11d	.	.	16d	+	*SUP3*
2a	+	+	7a	+	+	12a	+	+	17a	*sal4*	+
2b	+	*SUP3*	7b	*sal4*	*SUP3*	12b	*sal4*	+	17b	+	+
2c	*sal4*	*SUP3*	7c	+	+	12c	*sal4*	+	17c	+	*SUP3*
2d	*sal4*	+	7d	*sal4*	*SUP3*	12d	+	*SUP3*	17d	.	.
3a	+	+	8a	+	+	13a	*sal4*	+	18a	*sal4*	+
3b	+	+	8b	+	*SUP3*	13b	+	*SUP3*	18b	+	*SUP3*
3c	*sal4*	*SUP3*	8c	*sal4*	+	13c	+	+	18c	+	+
3d	.	.	8d	.	.	13d	.	.	18d	.	.
4a	*sal4*	+	9a	+	*SUP3*	14a	*sal4*	+	19a	+	*SUP3*
4b	+	*SUP3*	9b	*sal4*	+	14b	+	+	19b	*sal4*	+
4c	+	+	9c	+	*SUP3*	14c	+	*SUP3*	19c	+	+
4d	.	.	9d	*sal4*	+	14d	.	.	19d	.	.
5a	*sal4*	+	10a	*sal4*	*SUP3*	15a	*sal4*	+	20a	+	+
5b	+	+	10b	+	+	15b	*sal4*	+	20b	*sal4*	+
5c	+	+	10c	+	*SUP3*	15c	+	+	20c	+	*SUP3*
5d	.	.	10d	*sal4*	+	15d	+	*SUP*	20d	.	.

Table 11. *Germination of spores in tetrads from* [psi$^+$] *diploids heterozygous for an allosuppressor mutation*

Diploid no.	Allosuppressor mutation	*SUPQ5* locus	Numbers of tetrads in which so many spores germinated: 4	3	2	1	0	
540	*sal1.1*	*SUPQ5*/+	7	2	1	0	0	
555	*sal2.4*	*SUPQ5*/+	9	1	0	0	0	
556	*sal3.1*	*SUPQ5*/+	2	5	3	0	0	
602	*sal3.5*	*SUPQ5*/+	0	0	12	13	5	
573*	*sal3.5**	*SUPQ5*/+	4	3	7	2	4	
557	*sal4.1*	*SUPQ5*/+	0	0	8	1	1	
574	*sal4.2*	*SUPQ5*/+	0	1	31	4	4	(28 °C)
			3	12	4	0	1	(21 °C)
603	*sal4.2*	+/+	0	0	3	7	0	(28 °C)
616	*sal4.2*	+/+	0	0	8	0	2	(28 °C)
			3	3	2	0	2	(21 °C)
609†	*sal4.2*	+/+	7	2	1	0	0	
–	*sal5.3*	*SUPQ5*/*SUPQ5*	6	4	0	0	0	

* It was found that *sal3.5* did not segregate in these tetrads.
† All cultures proved to be [*psi*$^-$].

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(*b*) *Interaction with the* [psi$^+$] *factor*

The results of tetrad analysis of diploids containing allosuppressors in a [*psi*$^+$] background are recorded in Table 11. Here the interactions are different. It was found that *sal1.1*, *sal3.1* and *sal5.3* do not significantly affect the viability or vigour of [*psi*$^+$] strains, whether or not the *SUPQ5* mutation is present.

In contrast to the results with these four alleles, two alleles of *sal4*, *sal4.1* and *sal4.2* and one of *sal3*, *sal3.5*, interact with the [*psi*$^+$] factor to produce a lethal phenotype.

Six diploids involving these mutations were analysed. They are considered separately.

(1) *557*. This diploid was [*psi*$^+$] and heterozygous for the *sal4.1* mutation. Ten tetrads dissected yielded eight in which only two spores germinated, one in which one spore germinated and one in which none did. This is consistent with the segregation of a lethal mutation. All were found to be [*psi*$^+$]. The segregation of *sal4.1* was not assayed. *SUPQ5* segregated independently of the lethal phenotype.

(2) *574*. This diploid was [*psi*$^+$] and heterozygous for the *sal4.2* mutation. Forty tetrads were germinated at 28 °C. In one, three spores germinated, in 31, only two germinated, and in eight, less than two. The three cultures from the one tetrad in which three spores germinated, and the 18 cultures from nine other tetrads were mated by the single-cell method with strains of genotype *a* or *α* *SUPQ5* *sal4.2* *PNM2.19*. All clones were unsuppressed, indicating that none of these haploid segregants contained the *sal4.2* mutation. All segregants were [*psi*$^+$] and *SUPQ5* segregated independently of the lethal phenotype. The linkage of inviability with the *sal4.2* mutation suggests that *sal4.2* is lethal in a [*psi*$^+$] background.

Twenty tetrads were germinated at room temperature (21 °C). Among these, there were seven in which more than two spores germinated. The segregation of *sal4.2* was determined by the matings described above and it was found that many of the segregants carried the mutation. The presence of *sal4.2* coincided with an inability to grow at 28 °C.

(3) *616*. This diploid was also [*psi*$^+$] and heterozygous for the *sal4.2* mutation, but carried no suppressor (*supQ5*$^+$/*supQ5*$^+$). Segregation of lethality in tetrads was similar to that observed in diploid 574. When germinated at 28 °C, ten tetrads yielded eight viable diads and two in which no spore germinated. At room temperature, three or four spores germinated in six out of ten tetrads. No *sal4.2* alleles were found among the cultures germinating at 28 °C, but the mutation segregated normally in the set of tetrads germinated at room temperature. These *sal4.2* strains were unable to grow at 28 °C.

The results of the analysis of these two diploids demonstrates that *sal4.2*, which is conditional for growth in a [*psi*$^-$] background is also conditional for growth in a [*psi*$^+$] background, but the restrictive temperature is lower (28 °C instead of 34 °C).

(4) *602*. This diploid was [*psi*$^+$] and heterozygous for *sal3.5*. Among 30 tetrads, 20 of which were germinated at 28 °C and ten at room temperature, none yielded more than two viable spores. The 18 haploid segregants from nine diads were

mated by isolation of single zygotes with strains of genotype *a* or *α SUPQ5 sal3.5 PNM1.16*. No zygote clone was suppressed, indicating that no segregant contained *sal3.5*. Again, the linkage of inviability with the allosuppressor mutation shows that *sal3.5* is inviable in a [*psi*$^+$] background.

The two remaining diploids are exceptions which also, by being exceptional, illustrate the lethal interaction of these alleles with the [*psi*$^+$] factor.

(5) *573*. This diploid was [*psi*$^+$] and heterozygous for *sal3.5*. Among 20 tetrads, seven were found in which three or four spores germinated. All the segregants were mated with *SUPQ5 sal3.5 PNM1.16* strains, as described above. No segregant was found to contain *sal3.5*. This was confirmed by a ten-tetrad analysis of the four zygotes from one of the tetrads. Two, being homozygous *SUPQ5/SUPQ5* and heterozygous *sal3.5/+*, segregated two suppressed: two unsuppressed cultures in every tetrad. The other two, being double heterozygous, *SUPQ5/+*, *sal3.5/+*, segregated tetrads with 2:2, 1:3 or 0:4 suppressed: unsuppressed cultures.

It was clear that asci of diploid 573 do not have *sal3.5* segregating. It was isolated as a single zygote and the *sal3* locus probably became homozygous *SAL*$^+$*/SAL*$^+$ in part or all of the clone through mitotic crossing-over. This would remove one component of the lethal interaction and allow more than two spores to germinate in tetrads.

(6) *609*. The second exceptional diploid was of genotype

$$\begin{array}{llll} a & \underline{ade2.1} & \underline{sal4.2} & [psi^+]. \\ \alpha & ade2.1 & + & \end{array}$$

In seven of the ten tetrads dissected, all four spores germinated at 28 °C. Matings with *PNM*, *sal4* strains showed that *sal4.2* was segregating, as did the 2:2 segregation for temperature sensitivity. However, it was found that none of the segregants was [*psi*$^+$]. Clearly, this diploid, or those cells in the clone capable of sporulating, had lost the [*psi*$^+$] factor contributed by the [*psi*$^+$] parent of diploid 609.

This was the only diploid isolated using this [*psi*$^+$] strain (466/6a) as a parent in crosses with *sal3* or *sal4* strains, which sporulated. Twelve single zygotes and one mass-mating were tried. The implication is that it is only when either the allosuppressor or the [*psi*$^+$] factor is lost from cells of such diploids by mutation or other means, that normal sporulation may occur. It has also been noticed during the course of this study that it is a general property of [*psi*$^+$] diploids which are heterozygous for the *sal3.5* mutation that they cannot sporulate. Altogether eight diploids have been formed of this kind, and only three, of which diploid 573, whose behaviour was described above was one, sporulated at all. It is the only phenotype of any of the allosuppressor mutations which has been found to be dominant.

Recently, Rothstein, Esposito & Esposito (1977) showed that strong suppressor activity inhibits sporulation in *Saccharomyces cerevisiae*. Picard (1973) has shown a similar effect in *Podospora anserina*, and Sealy-Lewis & Casselton (1977) have observed sterility in dikaryons of *Coprinus lagopus* carrying two mutant suppressors.

(c) *Summary of the interactions of allosuppressors with other mutations*

The basic interaction of the allosuppressor mutations and that which is their principal phenotypic marker is with the *SUPQ5* suppressor mutation. The interaction allows the expression of the suppressed phenotype in the [*psi*$^-$] background in which it is normally silent.

In combination with Class I suppressors, which are able to suppress ochre mutations in a [*psi*$^-$] background, allosuppressor mutations lead to very reduced spore viability or, if germination succeeds, to poor growth.

In combination with the [*psi*$^+$] factor, three allosuppressor mutations, *sal3.5*, *sal4.1* and *sal4.2* prevent spore germination, the last only at temperatures of 28 °C and above. The others tested, *sal1.1*, *sal2.4*, *sal3.1* and *sal5.3*, have little or no effect on viability or growth when combined with [*psi*$^+$]. *Sal3.5* often has an adverse effect on the vigour of [*psi*$^+$] diploids in which it is heterozygous in that it reduces or abolishes sporulation.

Finally, for the most part, the allosuppressor mutations do not interact with each other. The exception is that alleles of *sal3* and *sal4* show a lethal interaction, whether or not the *SUPQ5* suppressor mutation is present. When the temperature-sensitive allele *sal4.2* is involved, the double mutant is only lethal at 28 °C and above. However, at 24 °C a novel phenotype is observed, namely the weak suppression of *ade2.1*, but not of *his5.2* or *lys1.1*.

(v) *Pleiotropic effects*

All allosuppressors isolated were tested for the ability to grow at two unusual temperatures, namely 12 °C and 34 °C. Nearly all alleles of *sal3* grew very poorly at 12 °C, if at all, compared with the strains from which the mutations were isolated. Five alleles of *sal3*:*sal3.1*, *sal3.4*, *sal3.5*, *sal3.7* and *sal3.56*, segregate in tetrads linked to the cold-sensitivity.

Two mutants, *sal3.56* and *sal4.2*, were found to be unable to grow at 34 °C. Tetrad analysis showed that the temperature-sensitivity of *sal3.56* segregated independently of the *sal* and cold-sensitive phenotypes, which are linked to each other. The temperature-sensitivity of *sal4.2* segregated with the allosuppressor phenotype. It is interesting to note that, apart from growth, all aspects of the allosuppressor phenotype of this mutant are temperature-sensitive. That is, the lethal interaction with Class I suppressors, with the [*psi*$^+$] factor and with *sal3* are remedied by incubation at 24 °C or below. At this temperature also, and even more markedly at lower temperatures, the interaction of *sal4.2* with *SUPQ5* is also reduced. *SUPQ5*, *ade2.1*, *sal4.2* [*psi*$^-$] strains are pink at 24 °C, red at 20 °C and below and at 20 °C and below, neither *his5.2* nor *lys1.1* are suppressed.

(vi) *Linkage*

With a single exception, no linkage has been observed between any of the allosuppressors, or between them and other markers segregating in various crosses; namely, mating-type, *his5*, *lys1*, *can1*, *ura3*, *SUP2*, *SUP3*, *SUP11* or *SUPQ5*.

However, it appears that the *sal3* and *PNM2* loci are linked. The segregation of the *PNM* phenotype was assayed by single-cell matings with *ade2.1 SUPQ5* [*psi*$^+$] strains. *PNM*$^-$ diploids are red, since they become [*psi*$^-$]; *pnm*$^+$ remain [*psi*$^+$], are suppressed and are white. Only nine tetrads from a diploid heterozygous for both *PNM2.19* and *sal3.5* have been analysed: all were of parental ditype constitution. A strain mutant at the second known *PNM* locus was crossed with strains mutant at each of the allosuppressor loci and tetrads analysed. All five allosuppressors segregated independently of it. The possibility that allosuppressor mutations may be allelic with antisuppressors (McCready & Cox, 1973) or with universal suppressors (Hawthorne & Leopold, 1974) is being investigated.

4. DISCUSSION

In strains of genotype *ade2.1*, *supQ5*$^+$, [*psi*$^-$] spontaneous mutations to adenine independence are due largely to mutations of the Class I super-suppressor type (Schuller & von Borstel, 1974). These are probably mutations in the third (5′) anticodon position in one or other of eight genes specifying tyrosine-inserting t-RNAs (Gilmore, Stewart & Sherman, 1971). In contrast, 90 % or more of spontaneous adenine-independent reversions in strains of the genotype *ade2.1*, *SUPQ5*, [*psi*$^-$], occur at only five loci, the allosuppressor loci described here. Reversions of this type occur spontaneously about 100 times more frequently than do the reversions in *supQ5*$^+$ strains. The high rate of mutation at these five loci suggests therefore that many sites within them may mutate to produce the change in phenotype. This in turn suggests that the loci may determine the structure of proteins.

Mutations at these five loci are recessive. Their effect is to cause an increase in the efficiency of suppression. This is true not only of the suppressor activity of the mutant *SUPQ5* locus, but also, apparently of the suppressor activity of Class I suppressor loci. This is indicated by the lethal interaction of allosuppressors with these suppressors.

The corollary is that it is the function of the *wild-type* products of these loci to *reduce* the efficiency of suppression by mutant t-RNAs. The frequency of read-through of ochre codons by Class I suppressors has been measured *in vivo* at about 10 % and by *SUPQ5* at less than 1 % in [*psi*$^-$] strains (Liebman *et al.* 1975). Thus in suppressed strains there is competition between the normal chain-termination apparatus and the altered t-RNA for the ochre codons in m-RNA. The competition is resolved in favour of chain termination by the activity of allosuppressor gene products.

There would seem to be three general ways in which these gene products could act. One is by maintaining a low level of t-RNA relative to chain-termination factors. In other words, allosuppressors may be genes producing repressors of t-RNA production. Since they all interact with both tyrosine-inserting suppressors and a serine-inserting suppressor, it is implied that they would be general repressors rather than specific to particular species of t-RNA. The second possibility is that they code for proteins required for efficient chain termination, for example release factors. Thirdly, to the extent that ochre codon reading by these suppressors may

demand wobble (Crick, 1966) these genes may code for proteins which reduce or restrict it.

A fourth possibility has to be discounted. It is that the mutant allosuppressors produce altered t-RNAs. They would be produced in such low levels or have such a low intrinsic efficiency of suppression that by themselves they would not produce a suppressor phenotype but combined with another weak suppressor, for example *SUPQ5*, they would. This cannot be reconciled with their lethal interaction with Class I suppressors. *SUPQ5* does not have a lethal interaction with any Class I suppressor. Even two Class I suppressors together do not show a lethal interaction: they merely grow more slowly (Gilmore, 1967).

It is not possible to discriminate between the first three theories without more information. However, the interactions of two of the allosuppressors, *sal3* and *sal4* with each other and with the [*psi*$^+$] factor strongly suggest that their site of action and that of the [*psi*$^+$] factor or its product is ribosomal rather than chromosomal. The lethal interactions of these three determinants are observed even in the absence of the product of the mutant *SUPQ5* gene. That is, the spores fail to germinate even when there is no known suppressor t-RNA present. Were the products of the two allosuppressor loci repressors of transcription of t-RNA genes or t-RNA maturation enzyme genes, it would follow first that at least two different repressors were required for all such operators the loss of either of which would lead to some lose of repression, and secondly that over-production of normal, non-suppressing t-RNA is lethal.

These are interesting possibilities, but it seems more plausible to suppose that the *sal3* and *sal4* gene products are proteins involved in determining the precision of translation at the ribosome. This is strongly supported by two observations concerning the temperature-sensitive *sal4.2* mutation. The first is that at a temperature which permits the double-mutant *sal3.5*, *sal4.2* to survive and grow, evidence is found of an unusual suppression pattern. Often, the *ade2.1* allele is not suppressed by weak suppressors, when the *his5.2* and *lys1.1* mutations are (Gilmore & Mortimer, 1966). In this double mutant the situation is reversed. There is very weak suppression of *ade2.1* but none of *his5.2* or *lys1.1*. The suppression occurs, of course, in the absence of any known suppressor mutation at a locus coding for tRNA. This implies a low level of mistranslation characteristic, for example, of the *ram* mutations found in both *Escherichia coli* and in yeast (Gorini, 1970; Hawthorne & Leupold, 1974). This in turn suggests that *sal3* and *sal4* code for proteins of the ribosomal complex. The second observation is the failure of *sal4.2* to grow at 34 °C, even in the absence of *SUPQ5*. This is also difficult to reconcile with the action of a defective repressor protein, but is readily accounted for if the gene product is required for protein synthesis.

If either gene product can be shown to be ribosome-associated, one involved with polypeptide chain assembly rather than with the production of amino-acyl t-RNAs, it follows that the others must be also. The same then follows for the [*psi*$^+$] factor or its product. The *sal4.2* mutation should provide the means for a direct biochemical approach to resolving this question.

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