A RECESSIVE LETHAL SUPER-SUPPRESSOR MUTATION IN YEAST AND OTHER Ψ PHENOMENA

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1. INTRODUCTION

SUPER-SUPPRESSOR mutations are readily isolated in certain auxotrophic strains of yeast by selecting for prototrophic variants. They arise spontaneously or after treatment with mutagens (Hawthorne and Mortimer, 1963; Hawthorne, 1969b; Inge-Vechtomov, 1965). Since, often, they are dominant, they can as readily be found in diploid as in haploid auxotrophs. All the evidence suggests that they act, as do the better-known amber and ochre suppressors in bacteria, through the misreading of mutant nonsense codons by means of altered amino-acid-accepting transfer RNA species (Gilmore and Mortimer, 1966; Gilmore, Stewart and Sherman, 1968).

Among a number of suppressors of adenine requirement arising in a hetero-allelic adenine-requiring diploid used in this laboratory was one, S_{Q2} which, in a tetrad analysis of the diploid strain, gave the results summarised in tables 1 and 2. Four facts stand out. First, a recessive lethal mutation

TABLE 1

The pattern of survival of spores in tetrads of the diploid prototroph Q2. The tetrads are classed according to the number of spores which germinated to form viable cells. Asci were dissected on YC or YNB medium and germinated at various temperatures, as shown

Temperature and medium	Number of tetrads in which so many spores germinated					Percentage germination	Number of spores	
	4	3	2	1	0		ad^-	AD^+
20° C., YC	0	0	9	1	0	48	19	0
25° C., YC	0	0	10	0	0	50	20	0
28° C., YC	0	0	56	1	0	50	113	0
34° C., YC	0	0	8	1	0	47	17	0
37° C., YC	0	0	8	0	0	50	16	0
37° C., YNB	0	0	9	1	0	48	19	0
Total	0	0	100	4	0	49	204	0

is segregating, since, in a majority of tetrads dissected, only two spores germinated to form viable colonies (table 1). Secondly, the adenine independence of the diploid is due to a suppressor mutation, since both alleles of the ad_2 locus segregate out in the haploid progeny. Thirdly, it is clear that the lethal is either identical with or very closely linked to the mutation causing suppression of adenine requirement in the diploids, since none of the segregants is adenine independent. Finally, the lethal mutation is centromere-linked: the digenic tetrad types formed by the segregation of this and of the tightly *c*-linked tr_1 marker (Hawthorne and Mortimer, 1960), are fifty-nine ditypes and eight tetratypes. The following is an account of a genetic analysis of this behaviour.

2. MATERIALS AND METHODS

(i) Strains

1. S_{Q2} arose in a diploid of genotype:

$$2n = \frac{a}{\alpha} \; \frac{me_2}{+} \; \frac{ad^o_{2,\,1}}{ad_{2,\,c}} \; \frac{hi_8 \; se_1}{+ \; +} \; \frac{tr_{1,\,1}}{+} \, .$$

The adenine-independent revertant of this diploid was named $Q2$. The $ad_{2,\,c}$ and se_1 markers were isolated in this laboratory. All the other markers originate in strains provided by Dr D. C. Hawthorne and Dr R. K. Mortimer.

2. Other markers used in this study were derived as follows: $hi^o_{5,\,2}$; $ly^o_{1,\,1}$ and $arg_{4,\,23}$ from JM32 and $hi_{2,\,1}$ from S723C, both provided by Dr R. K.

TABLE 2

The segregation of the lethal phenotype in relation to the other markers in the diploid. Ditype half-tetrads are of two kinds: either both spore-cultures are wild-type or both are mutant ($+$, $+$ or $-$, $-$). In tetratype half-tetrads, one of the two surviving spores is wild-type, the other mutant ($+$, $-$). Except for the segregation of mating-type, the results are given from sixty-seven tetrads analysed. The figures shown in the last line are those expected from a random assortment of a lethal gene and a marker and, in the last column, the probability that each marker so assorts is calculated from the χ^2 value using these figures

	Number of half-tetrads					
	$+, +$ or $ad_{2,\,c}, ad_{2,\,c}$ or a, a	$-, -$ $ad^o_{2,\,1}, ad^o_{2,\,1}$ α, α	$+, -$ $ad_{2,\,c}, ad^o_{2,\,1}$ a, α	Total	χ^2	P
Marker						
a/α	14	9	13	36	17·1	$<0·01$
ad_2	9	6	52	67	2·87	0·25
hi_8	17	7	43	67	4·66	0·10
se_1	13	10	44	67	0·43	0·80
me_2	18	8	41	67	5·38	0·07
tr_1	30	29	8	67	90·30	$\ll 0·01$
Expected	11·17	11·17	44·67	67·01		

Mortimer; S_D from X1687-20D, provided by Dr R. A. Gilmore. It is allelic with S_{11} in Hawthorne and Mortimer's classification (1968).

3. Various other super-suppressor mutations are briefly described. They were also provided by Dr R. A. Gilmore.

The mutations designated $ad^o_{2,\,1}$; $hi^o_{5,\,2}$ and $ly^o_{1,\,1}$ are suppressible by super-suppressors, and Hawthorne (1969a, b) reports that they are due to mutations to an ochre (UAA) codon. $tr_{1,\,1}$ is suppressible by another class of super-suppressors and is said to be due to an amber (UAG) mutation; it is, however, not suppressible by any of the suppressors described here. As an aid to following the discussions and descriptions, the three " ochre " suppressible alleles will be identified throughout the text by a superscript " o ".

The remaining symbols used are the conventional ones and are described in the 1966 supplement to the *Microbial Genetics Bulletin*. " S " indicates a dominant suppressor, and in this paper always designates a super-suppressor. Different super-suppressors are distinguished by subscripts.

Super-suppressors have been classified on the basis of their patterns of suppression (Hawthorne and Mortimer, 1968; Hawthorne, 1969a, b; Gilmore, 1966, 1967). In this paper, the classes referred to are those

defined by Gilmore since, where comparisons have been made, they were with his suppressors.

Mutations at the ad_2 locus confer a red coloration on the strains. Suppressed ad_2 mutations do not, and the strains are the normal white colour.

(ii) *Media*

YC, yeast complete (Cox and Bevan, 1962), gelled or not with agar, is the normal growth medium.

YNB is used as a minimal medium, with growth-factor supplements as required. It is made from Difco yeast nitrogen base without amino acids, with 2 per cent. dextrose and solidified with agar.

The sporulation medium used is that described by Hurst and Fogel (1964).

(iii) *Culture methods*

Standard bacteriological techniques are used in plating and subculturing. Single-cell isolations and tetrad dissection are carried out with a micromanipulator. Asci are prepared for dissection by treatment with snail enzyme (Johnston and Mortimer, 1959). Matings are performed by isolating zygote figures with a micromanipulator from mixtures of cells of opposite mating-types. Occasionally, such mating-mixtures are directly sporulated.

Nomenclature

Example: 198/6c is the culture which grew from the third spore (numbered *a-d*) in the sixth tetrad dissected from diploid 198. The nomenclature of the other strains follows the same practice.

3. EXPERIMENTS

(i) *Rescue matings*

The close *c*-linkage of the lethal mutation suggests that the lethality arises from a centromere defect which, at the time of meiosis or after causes misdivision, specifically, perhaps, a non-disjunction of one chromosome so that inviable nullisomic or disomic haploid cells are formed. The first step, then, is to test whether the lethal genome can be recovered and if it can, whether it is intact, or aneuploid. It is also recorded (Mortimer and Hawthorne, 1966) that the super-suppressor S_D is closely *c*-linked on chromosome VI.

It was decided to cross a lethal-carrying strain with two strains. One was of genotype: $228/3d = \alpha$, $ad_{2,\ 1}^{o}$, $hi_{2,\ 1}$; hi_2 being a *c*-linked marker on chromosome VI (Mortimer and Hawthorne, 1966). The other strain carried super-suppressible markers and was of genotype: $198/6c = a$, $ad_{2,\ 1}^{o}$, $hi_{5,\ 2}^{o}$, $ly_{1,\ 1}^{o}$, $arg_{4,\ 23}$, $tr_{1,\ 1}$.

To carry out a genetic analysis with a gene fatal to any haploid cell carrying it presents difficulties. However, a process of spore rescue by mating proved successful. A set of tetrads of the S_{Q_2} diploid was dissected and the spores moved to marked positions on a YC agar plate. A single haploid cell of the chosen rescuer strain was placed in contact with each spore in each tetrad. The paired cells were observed at intervals and a record kept

of the zygotes formed. As soon as the two mating figures in each tetrad could be identified, the other two pairs of cells (which must have been pairs of identical mating-type) were separated so that spore-cultures of each tetrad could be recovered uncontaminated by the rescuer strain.

Only one kind of tetrad can be rescued as a complete tetrad by this technique—that in which the lethal mutation segregates in coupling with a mating-type allele complementary to that of the rescuer strain. The technique is illustrated in fig. 1.
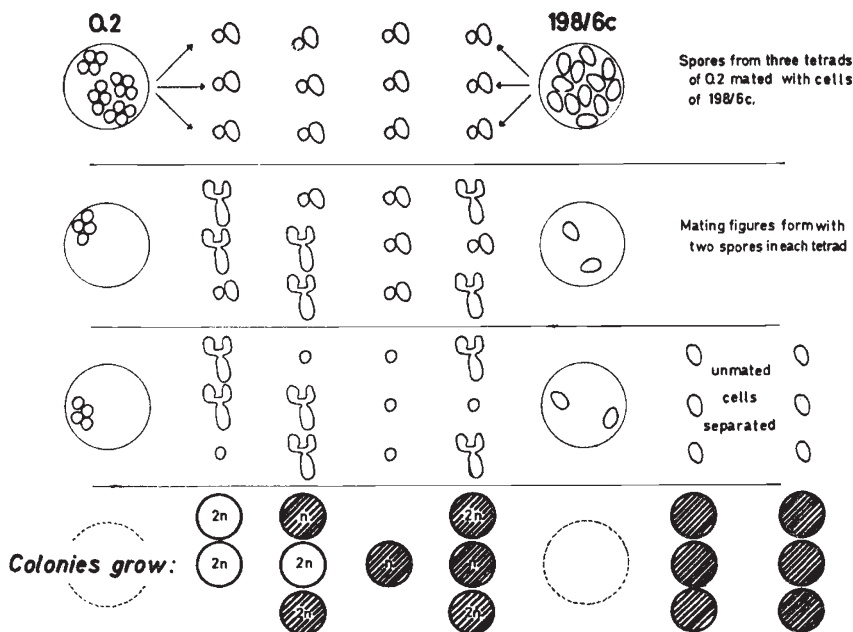

Fig. 1.—Rescue by mating. The figure shows three tetrads of the diploid Q2 dissected out and each spore paired with a cell from a culture of haploid strain 198/6c. The genotypes of the two strains are given in table 3, section A. The cells at two subsequent stages of the operation, and finally the colonies that grow from them, are shown. Adenine-requiring colonies are shown hatched, and the ploidy of the cultures is indicated.

Two such whole tetrads and ten other zygotes were recovered from the crosses with 198/6c and a number of zygotes from the crosses with 228/3d. Recovery was incomplete: there were not always two zygote figures formed in every tetrad.

Four adenine-independent (suppressed) zygote cultures, two from each set of crosses were sporulated and tetrads dissected. The germination in these tetrads is indicated in the general table of germinations (table 4, lines 1-4). It is clear that the lethal mutation is segregating. With two exceptions, all the surviving cultures were adenine requirers.

A further spore rescue operation was performed with the rescued zygote $Z6$, using 198/6c again as the rescuer strain. Two whole tetrads were recovered, together with a number of zygotes. A summary of the rescue operations is given in table 3.

TABLE 3

The phenotypes of strains formed by mating spores of S_{Q2}-carrying diploids with 198/6c. The segregation of markers in four complete tetrads is shown in this table. The first two tetrads are from the diploid Q2, the second two from Z6. The third part of the table summarises the important phenotypes of all the zygotes recovered in these experiments

(*A*)

$$Q2\ 2n = \frac{a}{\alpha} \cdot \frac{ad^{o}_{2,1}}{ad_{2,c}} \quad \frac{hi_8}{+} \quad \frac{se_1}{+} \quad \frac{me_2}{+} \quad \frac{tr_{1,1}}{+} \quad \frac{S_{Q2}}{+} \quad + \quad + \quad +$$

$$\times$$

$$198/6c = a \quad ad^{o}_{2,1} \quad + \quad + \quad + \quad tr_{1,1} \quad + \quad hi^{o}_{5,2} \quad ly^{o}_{1,1} \quad ar_{4,23}$$

			Phenotype			
Tetrad I	m.t.	ADE	HIS	SER	MET	TRY
a	a	−	+	+	+	−
b	a	−	−	−	−	−
c	zygote	+	+	+	+	+
d	zygote			—microcolony only—		

Tetrad II						
a	a	−	+	−	+	+
b	a	−	−	+	−	+
c	zygote	+	+	+	+	−
d	zygote	+	+	+	+	−

Also ten other zygotes, Z4–Z13. Six were AD^+, 4, ad^-. Tetrads of Z4 and Z6 were dissected with the results shown in table 4, lines 1 and 2. Z6 tetrads were also used in a rescue-mating experiment from which two complete tetrads were recovered (table 3 (*B*)).

(*B*)

$$Z6\ 2n = \frac{a}{\alpha} \cdot \frac{ad^{o}_{2,1}}{ad_{2,c}} \quad \frac{hi^{o}_{5,2}}{+} \quad \frac{ly^{o}_{1,1}}{+} \quad \frac{ar_{4,23}}{+} \quad \frac{tr_{1,1}}{+} \quad \frac{+}{S_{Q2}}$$

$$\times$$

$$198/6c = a \quad ad^{o}_{2,1} \quad hi^{o}_{5,2} \quad ly^{o}_{1,1} \quad ar_{4,23} \quad tr_{1,1} \quad +$$

			Phenotype			
Tetrad III	m.t.	ADE	HIS	LYS	ARG	TRY
a	zygote	+	+	+	−	not tested
b	zygote	+	+	+	−	not tested
c	a	−	+	−	+	not tested
d	a	−	−	−	+	not tested

Tetrad IV						
a	a	−	−	+	−	−
b	zygote	+	+	+	−	+
c	zygote	+	+	+	+	+
d	a	−	−	−	+	−

(*C*) *Summarised results*

Total tetrads dissected (Q2 and Z6 × 198/6c)	30
Number of zygotes formed with 198/6c cells	38
Number of adenine-independent zygotes	21
Number of adenine-dependent zygotes	17
Number of AD^+ zygotes from Z6 which were also HIS⁺, LYS⁺	15
Number of AD^+ zygotes from Z6 which required histidine or lysine	0

From these results we may draw several conclusions.

1. The suppressor of adenine requirement can be recovered from the spores of diploids in which adenine requirement is suppressed. In the four whole tetrads rescued, two of the spores carried the suppressor and the other two did not. Among the remaining diploids arising from zygote rescues, roughly equal numbers were requirers of adenine or adenine independent. As far as it can be judged from this result, therefore, the suppressor segregates as a mendelian factor. There is no regular non-disjunction or centromere malfunction of the chromosome on which it is situated.

2. The lethal mutation which is closely linked to it is recovered at the same time. This is shown by the six rescued adenine independent (AD^+) diploids which were analysed further. Segregation in them was almost invariably 2 ad-: 2 dead spore cultures (table 4, lines 1-6).

3. The tetrad analysis of these diploids showed that other markers from both parents always segregated. It must be concluded that these adenine-independent diploids arise from zygotes formed between the rescuer strain and a suppressor-carrying spore from the diploid, which would otherwise have died.

4. The suppressor of adenine requirement is a super-suppressor. In the rescued diploid $Z6$, three super-suppressible alleles are segregating: $ad^o_{2,1}$, $hi^o_{5,2}$ and $ly^o_{1,1}$. Rescue matings of spores of $Z6$ with 198/6c should often, therefore, be homozygous for one or more of these alleles. Tests for requirements of all the zygotes isolated show that all adenine-independent zygotes are also histidine and lysine independent, whereas adenine requiring zygotes may be requirers for either of these growth factors. Furthermore, the analysis of the whole tetrads of zygote $Z6$ shows that one of the rescued zygotes in tetrad 1 ($Z6/1a$ or $1b \times 198/6c$) must be homozygous for $hi^o_{5,2}$ and one in tetrad 2 must be homozygous for $ly^o_{1,1}$ (table 3). All four rescued zygotes in these two tetrads are, nevertheless, independent of lysine, histidine and adenine.

5. The lethal mutation is on chromosome VI. The zygotes formed by rescue matings of spores from S_{Q2} with the rescuer strain 228/3d would be expected to be of genotype:

$$2n = \frac{a}{\alpha} \frac{hi_{2,1}+}{+let^*} \frac{ad^o_{2,1}hi_8^{\pm}se_1^{\pm}}{ad^o_{2,1} + +} \frac{me_2^{\pm}}{+} \frac{tr_1^{\pm}}{+}.$$

Thus the segregation of $hi_{2,1}$ and the lethal mutation would form half-tetrads as follows:

	parental	non-parental	tetratype
	hi_2+	$hi_2\ let$	$hi_2\ let$
	hi_2+	$hi_2\ let$	hi_2+
	$+let$	$+\ +$	$+let$
	$+let$	$+\ +$	$+\ +$
giving	$2hi^-: 0$	$2HI^+: 0$	$1HI^+: 1hi^-: 0$

The numbers of each type observed in these two crosses, and in another in which both genes were segregating were: parental, 15; non-parental, 1; tetratype, 18. (Strain 233: 7P, 0N, 5T; strain 234: 4P, 1N, 12T; strain 243: 4P, 0N, 1T). This is taken to indicate linkage of hi_2 and the c-linked lethal mutation ($\chi^2_{P=N} > 12$; $p \ll 0.01$).

* let = lethal gene.

TABLE 4

The survival in tetrads from various diploids heterozygous for the suppressor mutation, S_{Q2}

Diploid no.	Mated strains	Percentage germination	Number of tetrads in which so many spores grew				
			4	3	2	1	0
1. Z4	$Q2$ spore × 198/6c	50	0	1[a]	8	1	0
2. Z6	$Q2$ spore × 198/6c	45	0	0	9	0	1
3. 233	$Q2$ spore × 228/3d	29	0	0	14	1	5
4. 234	$Q2$ spore × 228/3d	49	0	3[b]	14	2	0
5. 241	Z6 spore 1b × 198/6c	18	0	0	3	1	6
6. 242	Z6 spore 1a × 198/6c	43	0	1[c]	6	2	1
7. 244	233/4b × 228/1d	55	0	2	8	0	0
8. 243	234/17a × 228/1d	35	0	0	5	4	1
9. 356	234/17a × 233/12b	31	0	3[d]	53	9	35
10. 272	249/5b × 170/2c W (ψ^+)	53	0	4	4	1	1
11. 273	249/5b × 170/2c R (ψ^-)	100	10	0	0	0	0
12. 276	249/5a × 172/9b W (ψ^+)	58	3[e]	0	5	1	1
13. 277	249/5a × 172/9b R1 (ψ^-)	78	7	1	0	0	2
14. 278	249/5a × 172/9b R2 (ψ^-)	93	9	0	0	1	0
15. —	367/10c × 376/1a	30	0	0	6	0	4
16. —	367/2c × 376/1b	45	0	0	8	2	0
17. —	367/10c × 376/1c	50	0	0	10	0	0
18. —	367/2c × 376/1d	50	0	0	10	0	0
19. —	367/2c × 376/2a	0	0	0	0	0	10
20. —	367/10c × 376/2b	50	0	0	10	0	0
21. —	367/10c × 376/2c	48	0	0	9	1	0
22. —	367/2c × 376/2d	68	2[g]	5[g]	2	0	1
23. —	367/2c × 376/7a	55	2[h]	0	7[i]	0	0
24. —	367/10c × 376/7b	45	2[j]	0	5	0	3
25. —	367/2c × 376/7c	38	0	0	5	5	0
26. —	367/10c × 376/7d	43	0	0	7	3	0
27. —	367/10c × 197/2c (ψ^-)	83	7	0	1	1	1
28. —	367/10c × $ad^{o}_{2,1}$, a (ψ^+)	43	0	1	7	0	2
29. —	367/2c × $ad^{o}_{2,1}$, α (ψ^+)	30	0	0	5	2	3
30. —	367/2c × 197/2d (ψ^-)	90	9	0	0	0	1
31. 318	S_D × 193/1a (ψ^-)	88	8	1	0	0	1
32. 319	S_D × 172/9b R1 (ψ^-)	80	6	1	2	1	0
33. 323	S_D × 172/9b R2 (ψ^-)	93	8	1	1	0	0
34. 326	S_D × 172/9b W (ψ^+)	50	0	0	10	0	0
35. 324	S_D × 151/5c (ψ^+)	53	0	1	9	0	0

Notes—1. In Column 2, the strain carrying S_{Q2} is given first.

2. [a] All ad^-.

[b] In two of these there were micro-colonies which could not be subcultured. The third was 2 ad^- : 1 AD^+. The AD^+ is 234/17a (see ll. 8 and 9 and text).

[c] 2 ad^- : 1 AD^+. Not analysed further.

[d] 2 = all ad^-. 1 = 2 ad^- : 1 AD^+.

[e] S_{Q2} did not segregate in these tetrads.

[f] 1 = 1 AD^+ : 1 ad^-.

[g] 2 large colonies, 1 or 2 minute colonies in each tetrad.

[h] 2 AD^+ : 2 ad^-.

[i] 2 large : 2 minute in one tetrad.

[j] 2 large : 2 minute.

(ii) *The way of death*

Sets of asci were dissected, the spores moved to marked positions on agar plates and the plates incubated at various temperatures for 4 to 5 days, in which time two spores in most tetrads had grown, as is usual, to form

colonies of 2 mm. or more in diameter. The non-viable spores were in-
spected microscopically. A number of spores had germinated and divided,
some often enough to form microcolonies. Most of them remained undivided
or formed fewer than ten cells before growth ceased. Most of the single
cells and those in the very small groups were lysed or abnormal in appearance.
Cells in the larger microcolonies looked healthier on the whole, if somewhat
variable in size. However, all attempts to subculture these microcolonies
by re-streaking them on fresh YC agar plates failed. It is clear that whatever
causes death in these strains, its effects may be immediate or be delayed for
a few cell generations. It was also clear that the lethal effects are not modified
by differences in the temperature at which the spores are germinated (see
table 1). Table 5 shows the numbers of cells which grew from lethal-
carrying spores in one set of tetrads.

TABLE 5

The number of cells formed by each spore in a set of tetrads dissected from Q2 and germinated at 25° C.
The colonies were spread and counted after five days' incubation. Normal sized colonies were
not counted, and are recorded in the table as ' + '

| Tetrad | Spore no. | | | |
no.	a	b	c	d
1	71	+	+	1
2	308	129	+	+
3	7	+	+	37
4	+	9	13	+
5	13	+	44	+
6	296	2	+	+
7	+	344	+	50
8	22	+	8	+
9	15	+	+	1
10	+	29	51	+

(iii) *The viable suppressed haploid strains*

Tetrads from a diploid in which a lethal mutation is segregating should
never give rise to more than two viable cultures. However, among the two
hundred such tetrads so far described, there were a few found in which
three or four viable cultures were recovered. In some of these, all the
cultures were adenine dependent (ad^-) and they could therefore be ascribed
to irregular segregation or the occasional contamination of one tetrad by
spores from another, which is liable to occur when dissecting yeast asci
after cytase treatment. There was one tetrad, however, in which one of the
three cultures obtained was adenine independent while the other two were
adenine requirers. This culture was 234/17a. It seemed likely to be due to
a back-mutation or conversion of the lethal mutation which did not affect
the super-suppressor. There were two other cultures which were adenine
independent. These cultures were: 233/4b, one of two spores germinating
in a tetrad and Q2/13a, also one of two spores germinating. These may have
represented the results of recombination between the super-suppressor and
lethal mutations. Other explanations, including back-mutation of the
adenine mutation or, a second suppressor mutation and irregular segrega-
tion, were possible, so the first crosses were performed with a strain wild-type
for ad_2 (AD_2^+).

One of the adenine-independent cultures, Q2/13a, failed to mate with

any haploid tried. Although it also failed to sporulate, this may indicate that it was a diploid contaminant. In any case, no further analysis could be carried out. The other two cultures arose among the tetrads dissected from the rescues of $Q2$ spores by mating with 228/3d (table 4, lines 3 and 4). Both were crossed with 228/1$d = \alpha$, $hi_{2,\ 1}$, $tr_{1,\ 1}$, and asci dissected. The germination of the spores is shown in table 4, lines 7 and 8. In two of the twenty tetrads, three cultures grew, but in the rest, only two or fewer survived.   Among the cultures scored from cross 233/4$b \times$ 228/1d, ten were adenine requirers and ten adenine independent. This segregation pattern is consistent with what would be expected if a lethal mutation was segregating linked to a suppressor of adenine requirement. (That the adenine independence in 234/17a and 233/4b is due to a suppressor is proved by the segregation of adenine-requiring cultures from the crosses with the AD_2^+ strain. That the suppressor is linked to a lethal mutation is suggested by the equal numbers of adenine requirers and non-requirers segregating. If the non-viability was a random event or was due to an unlinked lethal mutation, the segregation expected is 3AD^+ : 1ad^-.   Summing over the two segregations, there are 14 AD^+ and 16 ad^- cultures; $\chi^2_{3AD:1ad} > 12$; P < 0·01.)   The segregation of the second cross 234/17$a \times$ 228/1d, is similar but less conclusive. However, this diploid was heterozygous for $hi_{2,\ 1}$, and it segreagted 9 hi^- and 1 HI^+ cultures, an indication of linkage between the lethal and hi_2^+ genes. In other words, the crosses seem to indicate that the original suppressor, S_{Q2}, is still present in these two haploid strains and that it is responsible for the adenine independent phenotype. It is also still associated with a gene which, in segregating from the diploids formed, behaves as a lethal.

    In order to confirm this interpretation, one of the two adenine independent haploids, 234/17a, was crossed with another strain, 233/12$b = \alpha$, $ad_{2,\ 1}^o$, $hi_{2,\ 1}$. The diploid was adenine and histidine independent.   One hundred asci were dissected. In all but three, only two or fewer spores germinated to form viable colonies, all being adenine requirers (table 4, line 9). In the three exceptional tetrads, three spores germinated. In one, all three cultures were adenine dependent (ad_2^-). In the other two, two spore-cultures were ad^- and one AD^+. One of these AD^+ cultures was diploid. The segregation of hi_2 and of mating-type in all the half-tetrads recovered was:

	hi_2			a/α		
(parental ditype)	−	−	31	α	α	9
(non-parental)	+	+	3	a	a	16
(tetratype)	+	−	19	a	α	27

    Clearly, the lethal mutation is again segregating, it is very closely linked to the suppressor since from only one of 400 spores tested did a viable adenine-independent culture grow; it is linked to hi_2 (there is a significant excess of parental ditypes, recombination is 28·7 per cent.) and it is centromere linked, since there is an excess of ditypes of the loosely centromere-linked mating-type gene ($\chi^2_{2D=T} = 5·13$; P < 0·05). Thus, in two matings of viable adenine-independent haploids, diploids were formed from which a lethal mutation segregated, and it is the same lethal that segregates in the original suppressed strain and in the diploids from which these two exceptional haploids were derived.   Therefore, whatever the reason for the

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viability of the two suppressor-bearing strains, it cannot be that the lethal mutation has been separated from suppressor mutation by recombination, mutation or gene conversion.

This result was not, however, consistently repeated. In some crosses involving one of the exceptional adenine-independent cultures, 234/17a, and strains carrying super-suppressible mutant alleles, no lethal gene segregated. The results are shown in table 6. It is clear that in three crosses

TABLE 6

The germination of spores and the segregation of markers in three diploids formed by mating 234/17a
(n = a, $ad^o_{2, 1}$, $se_{1, 1}$, $tr_{1, 1}$, S_{Q2}) with various complementary strains

Diploid no.	Number of tetrads in which so many spores germinated						Segregation of markers (number of tetrads)					
	4	3	2	1	0		$ad^o_{2, 1}$	se_1	$tr_{1, 1}$	$ar_{4, 23}$	$hi^o_{5, 2}$	$ly^o_{1, 1}$
249	9	1	0	0	0	4 : 0	*0	0	0	0	1	1
						3 : 1	0	0	0	1	4	2
						2 : 2	6	6	6	5	1	3
						1 : 3	0	0	0	0	0	0
						0 : 4	0	0	0	0	0	0
250	8	2	0	0	0	4 : 0	0	0	0	0	1	1
						3 : 1	0	0	0	0	4	7
						2 : 2	3†	8	8	8	3	0
						1 : 3	4	0	0	0	0	0
						0 : 4	1	0	0	0	0	0
252	18	1	0	0	1	4 : 0	0	0	not tested	0	not segregating	3
						3 : 1	0	0	—	0	—	11
						2 : 2	18	18	—	18	—	4
						1 : 3	0	0	—	0	—	0
						0 : 4	0	0	—	0	—	0

* Only six tetrads tested.
† A second, non-suppressible, unlinked adenineless mutation is segregating.

Note—All the diploids were homozygous for $ad^o_{2, 1}$ and heterozygous for S_{Q2} and for the other markers shown; except for Diploid No. 252, which is homozygous wild-type at the hi_5 locus, and heterozygous for the super-suppressor S_{Q5}.

germination is close to 100 per cent., but also that a super-suppressor segregated, suppressing both the $ad^o_{2, 1}$ allele, for which all the diploids were homozygous and the $hi^o_{5, 2}$ and $ly^o_{1, 1}$ alleles, for which they were heterozygous. The suppressor is c-linked, for of fourteen tetrads, thirteen showed ditype segregation of $tr_{1, 1}$ and the suppressor, and one a tetratype segregation 7P, 6N, 1T).

Thus it is observed that a super-suppressor, probably that originally isolated, S_{Q2}, is responsible for the adenine independence of the two exceptional cultures arising from tetrads from zygote-rescued spores. In some crosses the super-suppressor is closely linked, as before, with a lethal mutation, but in other crosses *involving the same super-suppressed haploid*, the lethal character fails to operate, and the super-suppressor is recovered in viable haploid cultures.

(iv) *The influence of ψ*

The third cross of table 6 is informative. Here, the haploid strain to which 234/17a was mated carries another super-suppressor, S_{Q5}. However, in this strain, S_{Q5} is inactive. As previously reported (Cox, 1965), the activity of S_{Q5} depends upon a cytoplasmic determinant, ψ. In ψ^+ strains, S_{Q5} suppresses super-suppressible alleles, but ψ^- strains remain auxotrophic whether S_{Q5} is present or not. Diploids formed by mating ψ^+ nd ψ^- haploids are ψ^+. Since S_{Q5} and S_{Q2}, the super-suppressor and lethal mutation under discussion, were both isolated in the same diploid strain, it was expected that all segregants would be ψ^+ and therefore that S_{Q5} would be active in the diploid and its haploid segregants. However, it is clear that, in the cross described in line three of table 6, only one active super-suppressor is segregating. The presence of S_{Q5} among the adenineless segregants is readily demonstrated by mating them with $ad^0_{2,\,1}$, ψ^+ strains. Any haploid carrying S_{Q5} then forms a diploid which is adenine independent, since S_{Q5} is active in this cytoplasmic background (*i.e.* $ad^0_{2,\,1}$, S_{Q5}, ψ^- (adenineless) $\times ad^0_{2,\,1}$, ψ^+ (adenineless)—$\rightarrow ad^0_{2,\,1}/ad^0_{2,\,1}$, $S_{Q5}/+$, ψ^+ (adenine independent). All the ad^- segregants from eighteen tetrads taken from this cross were cross-streaked with a and α $ad^0_{2,\,1}$, ψ^+ strains. By this test, in fourteen tetrads S_{Q5} was found to be present, but inactive, in one of the two ad^- segregants; in one tetrad it was present in both of them and in three tetrads it was present in neither. The inactive S_{Q5} therefore segregates independently of the active suppressor.

All these adenine-requiring segregants were also cross-streaked with a and α $ad^0_{2,\,1}$, S_{Q5}, ψ^- strains. All the diploids formed were adenine requirers, confirming that the diploid and its segregants were ψ^-.

By the same test, segregants from the cross 234/17a × 233/12b (table 4, line 9), in which the lethal phenotype was expressed, can be shown all to be ψ^+. The suppressor-carrying parent strain 234/17a is common to both these crosses.

These results show:

1. that a super-suppressor, S_{Q2}, appears to cause or to be linked tightly to a gene causing death in certain haploid strains;
2. that in other haploids, no death occurs due to this gene;
3. that those haploids in which the lethality is expressed segregate from diploids which are ψ^+;
4. that those which are viable are ψ^-; and
5. that when these ψ^-, viable, suppressed strains are crossed with other ψ^- strains, the suppressor segregates in a normal mendelian fashion, and no inviability is observed among the spores.

Since the original viable, suppressed strains arose among segregants from ψ^+ diploids, it is presumed that the acquisition of viability is due to a mutation from ψ^+ to ψ^-.

The role of the ψ factor in determining the expression of lethality of S_{Q2} has been confirmed in a number of crosses.

One set of crosses was made to check that the observed effects were a direct result of the $\psi^+ \longrightarrow \psi^-$ mutation. This mutation was first observed in three separate strains, carrying the S_{Q5} super-suppressor mutation (Cox, 1965). The ψ^+ and ψ^- subcultures of two of these strains, 170/2c and 172/9b were used as parents in crosses with haploids containing S_{Q2}. The results

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are shown in table 4, lines 10-14. The crosses involving the three independent ψ^- reversions show good viability, with several tetrads in which all four spores germinated. Only one suppressor appeared to be active, since adenine dependence: independence segregated 2 : 2. An excess of ditypes of adenine requirement and $tr_{1,\,1}$ was found in each cross (diploid 273 gave $3P$, $7N$, $0T$; diploid 277 gave $2P$, $5N$, $0T$; diploid 278, $5P$, $4N$, $0T$). This suggests that the suppressor expressed was the c-linked S_{Q2}. It was shown that S_{Q5} was segregating, although unexpressed, by crossing the adenine-requiring segregants with $ad_{2,\,1}^{0}$ ψ^+ strains. Some of the diploids formed in this way were white, and adenine independent. It is concluded that the diploids and all the segregants are ψ^-. This indicates that the S_{Q2}-carrying parent strain was also ψ^-.

The crosses involving the non-revertant, ψ^+ originals of these strains do not show the clear-cut recessive lethal segregation expected. In one case (diploid 276) this turned out to be because of a S_{Q2}^+ revertant clone present in the mating mixture from which the tetrads derived. In the other, one spore culture in each of the four tetrads in which three spores germinated grew, but only slowly. Plate I shows the tetrads derived from diploids 272 and 273, illustrating the ψ effect on the expression of lethality.

In both 272 and 276 tetrads, S_{Q5} segregated and was expressed as a suppressor, confirming that both diploids and segregants were ψ^+.

An attempt was also made to show that the determinant of the expression of lethality by S_{Q2} was, like ψ, not inherited in a mendelian pattern. A cross was made between a ψ^+ and a ψ^- strain, and tetrads dissected out. All the cultures from three of these tetrads were mated with viable haploid strains containing S_{Q2}. The results are shown in table 4, lines 15-26. With one or two exceptions, S_{Q2} plainly behaves as a recessive lethal in all these crosses. The crosses with the parent strains are shown in lines 27-30 of the same table.

Table 7 summarises the expression of the lethal character of S_{Q2} in ψ^+ and ψ^- strains. These include the following kinds of mating, described below. The carriers of S_{Q2} are of three kinds or classes:

I. Segregant spores of the original suppressed diploid rescued by mating, as described in section (1) above (seven strains).

II. Exceptional viable suppressed haploids (ten strains).

III. Descendants of these exceptions obtained from crosses between them and ψ^- strains (seven strains).

Cells of the first class are expected to be ψ^+, those of the last two, ψ^-. Their mating partners have been:

Class I crossed with ψ^+ ($228/3d$, $198/6c$).

Classes II and III crossed with:

 A. segregants of the original diploid, $Q2$—expected to be ψ^+;

 B. the original ψ^+ and ψ^- strains;

 C. parents and relations of these original strains—expected to be ψ^+;

 D. segregants from $\psi^- \times \psi^-$ matings—expected to be ψ^-;

 E. segregants from $\psi^+ \times \psi^-$ matings—expected to be ψ^+;

 F. JM32—found to be ψ^- (Cox, 1965);

 G. sibs of Class (III) carriers—expected to be ψ^-.

Some matings, therefore, are expected to give rise to ψ^+ diploids. These are all those involving Class (I) carriers of S_{Q2}, and all Class (II) and (III)

carriers mated with types A, C, E and ψ^+ forms of type B. Segregations from these diploids are recorded in the top half of the table. The other

TABLE 7

A summary of the survival of spores in tetrads from all the diploids analysed in this study in which S_{Q_2} mutation was segregating. The table includes notes on those segregations which are exceptions to the general rule that S_{Q_2} is lethal in ψ^+ strains but not in ψ^- strains. The survival of spores in these exceptions is not recorded in the main body of the table, but in the notes below. Data already given in table 1 is excluded altogether

Source of S_{Q_2}*	Type of mating*	No. of diploids analysed	Number of tetrads with so many spores germinating					No. of exceptions
			4	3	2	1	0	
(i) ψ^+ *Diploids:*								
I	—	7	1	6	48	13	16	none
II	A	2	0	3	62	10	35	none
	C	8	0	3	33	9	5	3[a]
		(5 recorded)						
	E	7	0	3	33	4	1	2[b]
		(5 recorded)						
III	A	2	0	1	18	0	0	none
	B	2	0	4	9	2	2	none
	C	4	0	4	22	3	5	none
	E	14	0	0	100	13	7	2[c]
		(12 recorded)						
Total ψ^+ Diploids		46	5	29	304	54	74	7
		(39 recorded)						
(ii) ψ^- *Diploids:*								
II	D	9	52	6	4	1	18	1[d]
III	D	6	51	6	4	2	4	none
	B	3	26	1	0	1	2	none
	G	3	25	2	0	1	2	none
	F	1	10	0	0	0	0	none
Total ψ^- Diploids		22	164	15	8	5	26	1
		(21 recorded)						

(iii) *Notes on exceptional segregations:*

[a] Matings of three viable suppressed haploid strains from a $234/17a \times \psi^+$ mating. All segregants were ψ^+.

a_1. $292/3a \times \psi^+$:	0	1	8	1	0
a_2. $292/5a, 292/7a \times \psi^+$:	18	0	2	0	0

[b] Suppressed segregants from a_2 above.

$364/7c, 366/4c \times \psi^+$:	10	6	4	0	0

[c] Most S_{Q_2} segregants grew very slowly (see table 4, lines 22 and 23).

$367/2c \times \psi^+$:	4	5	9	0	1

[d] S_{Q_2} behaved as a recessive lethal, although the segregants were all ψ^-.

$234/17a \times \psi^-$:	0	1	13	2	1

* An explanation of the symbols in these columns is given in the text. The origin of the strain 234/17a is shown in table 4, line 4, and is also mentioned in the text.

matings are expected to give rise only to ψ^- diploids, and segregations from them are recorded in the bottom half of the table.

If the segregations from the ψ^+ diploids are considered first it will be seen that among 437 tetrads recorded from 40 diploids, only two spores germinated in 304 (70 per cent.) of them, and in only 34 (8 per cent.) did more than

two germinate. The lethality was always, in the 2 : 0 segregations, linked with the S_{Q2} suppressor. Thus the overall pattern is that expected if S_{Q2} is a recessive lethal mutation, with a minor fraction of exceptions. These will be described below.

The segregations from ψ^- diploids show no sign of the lethal phenotype of S_{Q2}. Among 218 tetrads from 22 diploids, 164 (75 per cent.) gave rise to four viable cultures. The viability of S_{Q2} haploids was no different from that of S_{Q2}^+. There was one exception (table 7, footnote d).

The exceptions among ψ^+ diploids to be considered are, firstly, those tetrads in which three or more spores gave rise to viable cultures. Some of these were trivial, in that the extra culture did not carry the S_{Q2} suppressor gene, and therefore would not be expected to be inviable.

Others were suppressed. Almost invariably, such cultures were very slow to germinate and grow, giving rise to minute colonies compared to their sibling spore-cultures. However, regardless of their vigour, they all proved on crossing, to be ψ^-, while their sibs remained ψ^+. The occurrence of these kinds of exceptional segregant is important in relation to the effects of the ψ factor and the reasons for the lethal phenotype.

Secondly, one ψ^+ diploid, 292 (footnotes a and b, table 7) produced a number of normally viable suppressed haploid strains. All the non-suppressed sibs of these cultures were ψ^+. One of the viable suppressed segregants gave rise to a characteristic recessive-lethal segregation on crossing to a ψ^+ strain (table 7, footnote a_1). Two others, however, showed no lethality of the suppressor in such a cross (footnote a_2), nor in second-generation crosses with ψ^+ strains (footnote b). All the non-suppressed sibs of these second and third generation, viable, suppressed segregants were shown to be ψ^+. It must be concluded that, in all likelihood, these S_{Q2}-carrying segregants are also ψ^+, but not fatally affected by the lethal gene. At present, the best explanation for this exceptional diploid is that a dominant modifying mutation has occurred which negated the lethal effects of S_{Q2}, in the original haploid and in segregants from crosses with it, without affecting the segregation of the ψ^+ factor.

The single exceptional segregation among ψ^- diploids (table 7, footnote d) is still unexplained. In a later repeat of this cross, germination was 95 per cent. $(8-2-0-0-0)$.

(v) *Behaviour of* S_D

S_{Q2} was found to be closely c-linked and linked to hi_2. This puts it very close to the super-suppressor S_D originally isolated by Hawthorne and Mortimer (1963). The identity of the two has not been established by a cross, but it has been found that S_D is also a recessive lethal in ψ^+ and not in ψ^- strains (table 4, lines 31-35).

(vi) *The isolation of more recessive lethal super-suppressors*

A small search was conducted to explore the occurrence and distribution of lethal super-suppressors in yeast. A ψ^+ diploid strain was constructed having the following genotype:

$$2n = \frac{ad_{2,1}^o}{ad_{2,1}^o}, \frac{hi_{5,2}^o}{+}, \frac{ly_{1,1}^o}{ly_{1,1}^o}, \frac{hi_{2,1}}{+}, \frac{tr_{1,1}}{+}, \psi^+.$$

Cells were plated on YC medium, and separate suspensions made of thirty-two colonies which grew. These suspensions were separately spread on YNB plates, and the plates irradiated with 1000 ergs/mm.² light at 260 nm. A single revertant colony was picked from each plate, each culture sporulated, and tetrads dissected. The results of the tests for segregations of the various markers are summarised in table 8. The suppressors are identified as alleles of S_D (S_{Q2}) if they show significant linkage with both the hi_2 locus and the centromere, using $tr_{1, 1}$ as a marker of centromere segregation.

Of the thirty-two revertants of independent origin:

2 could not be tested because they failed to sporulate;

1 showed an independent reversion of the ly_1 requirement and may not, therefore, be due to a super-suppressor mutation;

2 turned out still to require lysine: they may have grown originally as a result of some cross-feeding on the selection plate;

16 were super-suppressors which showed no loss of viability, and no indication of linkage to the hi_2 locus;

1 No. 26, segregated a recessive lethal independently of the super-suppressor mutation. The suppressor but not the lethal phenotype, in this strain is linked to hi_2, but probably not to the centromere. It may or may not be allelic to S_D: an inversion of the region could account for the data, which is too scanty to indicate whether hi_2 is significantly more closely c-linked than usual. Another suppressor, S_6 is known, distal and linked to hi_2 (Hawthorne and Mortimer, 1968);

1 reversion No. 28, is due to a super-suppressor which shows a reduced viability, but is not linked to either a centromere or the hi_2 locus;

5 reversions Nos. 22, 23, 24, 27 and 30, are due to suppressors which, since they show significant ($P < 0.05 > 0.01$) linkage to hi_2, may be allelic with S_D. Only one, No. 27, shows any reduction in viability;

4 by their close c-linkage and highly significant ($P < 0.01$) linkage to hi_2, either in coupling or repulsion, are likely alleles of S_D. *Two* are recessive lethals (Nos. 14 and 32) and *two* have a very greatly reduced viability in haploids (Nos. 15 and 31).

From these results we may conclude that recessive lethal super-suppressors do recur; that the only locus which has shown complete lethality in haploids is the S_D locus; that it is possible, however, that alleles of S_D may occur which are not lethal or show merely a very reduced viability and that at least one other super-suppressor (revertant No. 28) is associated with a very reduced viability, though whether by linkage or by gene action is not shown.

(vii) *Reversions of* S_{Q2}

Six independent reversions of the S_{Q2} allele of S_D to amber-specific suppression have been isolated in a haploid, ψ^- strain. That they are due to mutations of the S_{Q2} mutant locus itself and not to mutations at other loci has been demonstrated by complementation and recombination analyses. All six have been mated with ψ^+ strains and asci dissected. Germination was found to be normal in all six amber versions of S_{Q2} (in 37 tetrads, all

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TABLE 8

A summary of the tetrad analyses of thirty-two adenine- and lysine-independent revertants of a ψ^+ diploid homozygous for ad_2^0, $_1$ and ly_1^0, $_1$ and heterozygous for hi_2 and the c-linked marker, $tr_{1,1}$

	Number of tetrads in which so many spores germinated					Number of Segregants of phenotype		Linkage		S-centromere		
Revertant number	4	3	2	1	0	ad^-	AD^+	S-lethal	S-hi_2	DT	TT	Notes
1	10	1	0	3	6	24	22	—	n.s.	7 : 3		A
2	2	0	1	2	5	4	4	—	n.s.	1 : 1		
3	1	3	0	0	6	6	6	—	n.s.	—		
4	6	1	1	0	2	17	20	—	n.s.	—		
5						too few asci						
6	7	1	2	0	0	14	14	—	o	—		
7	0	1	3	0	6	5	3	n.s.	n.s.	—		
8	0	0	1	2	7	3	1	n.s.	n.s.	—		
9	7	0	1	0	2	14	14	—	o	3 : 3		
10	0	0	6	1	3	1	4	n.s.	o	—		
11	6	2	1	0	1	14	10	—	n.s.	1 : 4		
12	9	2	0	4	5	23	19	—	o	6 : 2		
13	7	2	1	0	0	14	14	—	o	0 : 7		
14	0	3	47	9	1	112	0	complete	$++^c$	38 : 2		B
15	0	15	15	8	11	57	9	$++$	$++^c$	22 : 5		B
16	6	1	2	1	0	12	12	—	o	3 : 3		
17	0	0	4	5	1	2	6	o	n.s.	2 : 2		
18	7	2	1	0	0	17	11	—	o	0 : 6		
19	4	4	0	0	2	16	11	—	o	22 : 1		
20	5	3	1	0	1	10	10	—	o	—		A
21						no asci						
22	6	4	2	1	7	17	15	—	$+^r$	2 : 1		C
23	6	2	0	1	7	13	11	—	$+^r$	3 : 1		C
24	2	0	1	3	4	7	6	—	$+^r$	—		C
25	6	3	0	0	1	10	10	—	o	2 : 3		
26	0	0	42	10	8	45	39	oo	$++^r$	6 : 7		D
27	0	6	2	2	0	15	5	$+$	$+^c$	—		C
28	2	13	21	15	1	45	12	$+$	oo	5 : 6		E
29	7	3	0	0	0	12	12	—	o	2 : 4		F
30	0	1	6	1	2	9	6	n.s.	$+^r$	3 : 2		C
31	0	10	38	5	7	92	8	$++$	$++^r$	33 : 9		B
32	0	0	14	31	10	58	0	complete	$++^r$	8 : 2		B

Symbols: $+$, or $++$: linkage indicated at the 5 per cent. or 1 per cent. significance levels, respectively. " r " or " c " indicate whether the gene is in coupling or in repulsion with $hi_{2,1}$. o, or oo: non-linkage is indicated at the 5 per cent. or 1 per cent. levels, respectively. The null hypothesis is that S_D recombines with hi_2 in 30 per cent. of single-strands. n.s. = not significantly linked.

Notes:

Types found	Number	
A	2	All lys^-. Possibly not a suppressor
B	4	A recessive lethal S_D, or an allele of S_D with very reduced viability.
C	5	A possible non-lethal allele of S_D.
D	1	The suppressor was non-lethal, linked to hi_2 (12·5 per cent. recombinants) but not c-linked. The lethal mutation is independent of the suppressor, of hi_2 and the centromere.
E	1	A suppressor with very reduced viability, not an allele of S_D.
F	1	Lys→LYS⁺ independently reverted.
G	16	Viable suppressors, not alleles of S_D.
H	2	Not analysed.

four spores germinated; in 37 tetrads, three germinated; in 4, two germinated; in 5, only one and in 7 tetrads, no spores germinated; the overall percentage germination is 75 per cent.). In other words, the amber form of S_{Q_2} is not a recessive lethal when selected in ψ^- strains and transferred to ψ^+ cytoplasm. No attempt has yet been made to select amber-specific versions of S_D or S_{Q_2} in ψ^+ diploid strains.

4. Discussion

A super-suppressor which is lethal to most haploid cells has been isolated in a diploid strain of yeast. The lethality, but not the suppressor activity, is modified when the suppressor is included in yeast strains of the " ψ^- " type. " ψ^+ " and " ψ^- " strains are differentiated by their ability or not to express the activity of another super-suppressor, S_{Q_5} (Cox, 1965). The $\psi^\pm$ difference is inherited cytoplasmically.

Before going on to discuss the possible reasons for this occurrence, it is worth noting some other effects of the ψ^+/ψ^- character difference on cells. In cells with no super-suppressors, the ψ^+ or ψ^- character makes no difference to the growth of the cultures under various culture conditions, nor to the expression of genes, nor to their sensitivity to ultraviolet light, as measured by mutation or survival. Nor does the ψ status have any influence on the expression of two Class III super-suppressors (S_η and S_θ, Gilmore, 1966). That is, alleles suppressible by these suppressors continue to be suppressed whether the strains are ψ^+ or ψ^-.

However, if a Class I super-suppressor is present, the ψ status may have an effect. Two of these have already been noted: (1) the Class I super-suppressor S_{Q_5} does not suppress any suppressible allele in ψ^- strains; (2) many specifically ochre-suppressing alleles of the S_D locus are lethal in haploid ψ^+ strains, but not lethal in ψ^- strains, even though they continue to suppress ochre mutations.

A third effect should also be briefly described. Four other Class I, set 1 super-suppressors, S_α, S_β, S_γ, and S_δ were provided by Dr Gilmore (Gilmore, 1966). These were mated with ψ^+ and ψ^- strains. All zygotes from matings with ψ^- grew and sporulated, and in all of them the $ad^O_{2,\ 1}$ mutation and other ochre mutations were suppressed. However, of forty-six zygotes formed between cells of these cultures and cells of ψ^+ strains, only one grew normally. Most of the others (twenty-three) formed only minute colonies (200 μm after six days' growth: single zygotes normally form colonies 1000 μm in diameter after two days). Sixteen zygotes lysed immediately, or after a few divisions and the remainder grew only slowly. None of the cultures which grew was able to sporulate. A similar effect had been noted earlier with revertants of a ψ^- strain JM32. ($n = a$, $ad^O_{2,\ 1}$, $hi^O_{5,\ 2}$, $ly^O_{1,\ 1}$, $arg_{4,\ 23}$ ψ^-.) These were selected after UV treatment of individual colonies re-spread on YNB supplemented with arginine and lysine. They were therefore simultaneously adenine and histidine independent due, presumably, to super-suppressor mutations of Gilmore's Class 1. Twenty-seven revertants were mated with a ψ^+ strain and fifty-four zygotes isolated by micromanipulation. Seven grew normally, ten grew slowly, thirty-one formed colonies of 100 cells or fewer after six days and six lysed. The strain of S_D provided by Dr Gilmore also tends to form non-viable zygotes in ψ^+ crosses, but the effect is not so extreme.

We thus have a complex of effects of the ψ factor in combination with

Class 1 super-suppressors which can best be summarised in the form of a table (table 9).

In considering the association of a recessive lethal phenotype with alleles of S_D three general explanations occur.

1. A simultaneous, closely linked mutation occurs in a " vital " gene. This may take the form of a second, coincidental, point mutation, or the lethal and suppressor phenotypes may be due to a single deletion.

2. The mutation giving the suppressor phenotype, through a structural rearrangement, affects the behaviour of the neighbouring centromere.

3. The suppressor phenotype itself affects the viability of the cell. There is also the possibility that the suppressor is lethal because it is due to a mutation in a unique t-RNA species.

TABLE 9

A summary of the effect of the ψ factor on various super-suppressors in haploid and diploid strains of yeast

Suppressor	ψ^+		ψ^-	
	n	$2n$	n	$2n$
S_{Q5}	ochre	ochre	nil	nil
S_D, S_{Q2} (ochre)	lethal	ochre	ochre	ochre
$S_{\alpha, \beta, \gamma, \delta}$ and Class I (ochre)	?	lethal	ochre	ochre
S_{Q2} (amber)	amber	amber	amber	amber

The possibility of coincident, linked, point mutations is rendered unlikely by the following observations.

1. With all six likely repetitions of the ochre-suppressing mutation at the S_D locus, a recessive lethal or sub-lethal phenotype was associated.

2. In tetrad analysis, a number of haploid cultures were obtained in which the lethal phenotype had been lost and the suppressor phenotype retained. This could have been due to mutation or recombination separating determinants of the two phenotypes. However, further analysis showed that in every case but one, the recessive lethal phenotype was restored when the suppressed haploids were crossed to ψ^+ strains. One spore culture could be interpreted as being due to such a separation of the two phenotypes, but other interpretations are also possible.

3. Six independently isolated reversions of S_{Q2} to an amber-specific suppressor were accompanied by a loss of the recessive lethal phenotype. The frequent recurrence of such an event is unlikely if it requires the simultaneous reversion of two point mutations or, indeed, the reversion of a deleted region.

The second possibility, that a structural rearrangement leads both to the suppressor phenotype and the derangement of centromere function would require that this derangement did not affect centromere behaviour in diploid mitosis, since the suppressor is stably inherited in diploids; it has no great effect on meiosis, since the suppressor can, in most cases, be recovered from haploid spores by " rescue matings ", and did not affect it in ψ^- strains.

Either of these explanations is conceivable if a rather unconventional idea of the nature of super-suppressor mutations is accepted. However, since it

is reported that eight Class 1 super-suppressors are genes which cause the insertion of tyrosine at UAA codons in messenger RNA (Gilmore *et al.*, 1968; Sherman, Stewart, Parker, Putterman, Agrawal and Margoliash, 1970), it seems reasonable to suppose that they, like the SU_{III} suppressor in *E. coli*, are due to base-substitutions in the anti-codon regions of genes determining the structure of tyrosine-accepting t-RNA species (Goodman, Abelson, Landy, Brenner and Smith, 1968). Hawthorne (1969*b*) presents evidence and arguments why S_D should also be the result of mutations affecting a single base-pair in the anti-codon of tyrosine-accepting t-RNA's. If this is true, then an explanation of the properties of Class 1 super-suppression described here must be found in terms of gene action, that is of the effect of suppression itself.

This evidence also confirms that S_D is not unique, but one of a number of genes determining tyrosine-accepting t-RNA species. If it has a unique function, it cannot be its amino-acid specificity. Recently, Soll and Berg (1969) reported a recessive lethal amber suppressor in *E. coli* which appeared to be due to a mutation in a t-RNA^{gln} gene that is not unique. They suggested that the lethality might be owed to the major role normally played by the t-RNA specified by this gene in protein synthesis. Such a situation could explain the lethality of S_D in haploid yeast. However, unlike the *su*7 mutation, S_D does not appear to be a very efficient chain termination suppressor. Sherman *et al.* (1970) have found that Class 1 super-suppressors permit the completion of *iso*-1-cytochrome *c* protein from ochre-stopped cy_1 genes with at most 5 per cent. efficiency. As in *E. coli*, amber suppressors are more efficient. The discrepancy, it was originally suggested (Brenner, Stretton and Kaplan, 1966) could be a requirement of survival if the ochre codon were the normal chain-terminating codon: too efficient a misreading would have debilitating effects on normal protein synthesis. No such restriction need operate on the efficiency of amber suppression.

However, the source of the inefficiency is not understood. In *E. coli* it may be intrinsic, a property of the anticodon.

Amber and ochre alleles of a number of *E. coli* suppressors exist (Ohlson, Strigini and Beckwith, 1968; Person and Osborn, 1968), and the ochre suppressing alleles are derivable from amber by single base-pair mutagenesis. Ohlson *et al.* have been able to show that the change from amber to ochre suppression is accompanied in every case by reduction in the efficiency of suppression. Carbon and Curry (1969) have been able to show that missense-suppressing transfer RNA's have an intrinsic inefficiency in the sense that their rate of enzymic aminoacylation is altered.

An early suggestion by Brenner *et al.* (1966) was that the new ochre-recognising t-RNA in suppressed strains had to compete at the codon with an ochre-recognising t-RNA normally present and concerned in chain-termination. However, a study of the factors involved in chain termination in *E. coli* by Capecchi (1967), Scolnick, Tomkins, Caskey and Nirenberg (1968) and by Milman, Goldstein, Scolnick and Caskey (1969) has failed to demonstrate that t-RNA is involved at all. Indeed, the proteinaceous R-factors appear to recognise all three nonsense codons equally well, R_1 being specific for UAA and UAG and R_2 for UAA and UGA. There do not appear to be any results from the *in vitro* assay system which suggest that competition for UAA might be greater than for the others.

Another way of achieving the inefficiency would be through an in-

dependent gene modifying the mutant t-RNA, or, more likely, affecting its enzymatic modification. In *E. coli* this would have to be present in all strains used for genetic studies for, unless it were, it could not be transferred with every ochre suppressor in crosses, and it would therefore be impossible to transduce some ochre suppressors. The same restriction applies to yeast in which ochre suppressors would otherwise be lethal in half or more of the segregants from some crosses.

It is suggested that the phenomena described here are best explained if it is assumed that ochre suppression itself is potentially lethal when it becomes too efficient. The efficiency can be modified by secondary mutations and is also directly affected by, among other factors, the relative concentration of the mutant allele. Thus alleles of S_D are lethal when present in a certain concentration per unit of protein being synthesised (in haploids) but not in half that concentration (in diploids). The effect of the ψ status of the cell is to vary this efficiency, the efficiency, that is, of chain termination, which it does proportionately for all the tyrosine-inserting ochre suppressors. Let us suppose that in ψ^- strains, ochre suppressors only complete chains one-tenth as often as they do in ψ^+ strains. Then considering each kind of suppressor in turn, the S_{Q5} mutant is efficient enough to be expressed as a suppressor in ψ^+ strains, but at one-tenth efficiency, in ψ^- strains, no suppression is observable; S_{Q2} and other S_D alleles are efficient enough to suppress at both levels. In ψ^+ haploids, however, the efficiency is too great and therefore lethal, whereas at one-tenth the efficiency, in ψ^- strains, the cell's metabolism is unaffected; and similar differences occur in ψ^+ and ψ^- zygotes heterozygous for Class 1, set 1 super-suppressors.

This interpretation stems principally from a consideration of the nature of the exceptions observed to the rule that S_{Q2} behaves as a recessive lethal. They do not arise as reversions of the original suppressor-lethal mutation. Rather they arise as the result of a mutational event in an independent determinant, of a kind which modifies the phenotype. In suitable crosses, the modifier can be removed and the original phenotype fully recovered. It would seem that in yeast, one or more such modifying activities is a necessary condition for the survival of cells having certain ochre-recognising t-RNA species.

In the examples described here, most of the mutations have occurred in the cytoplasmically inherited ψ determinant. The exception described in a footnote of table 7 may be an example of a mendelian modifier affecting specifically the S_D gene.

A detailed consideration of the mode of action of such modifiers is not due here. The activity of enzymes which alkylate or otherwise alter bases in t-RNA; activating enzyme specificity; ribosomal modifications which affect the efficiency of codon-anticodon recognition; m-RNA folding or the regulation of the rate of synthesis of t-RNA's; any of these could be involved. Whatever the mechanisms, one at least has to be reconciled with the mode of inheritance of the ψ determinant.

5. SUMMARY

1. This paper is an account of the isolation in a diploid strain of yeast of an ochre-mutation suppressor, S_{Q2}, which is then segregated in meiosis as a recessive lethal.

Plate I
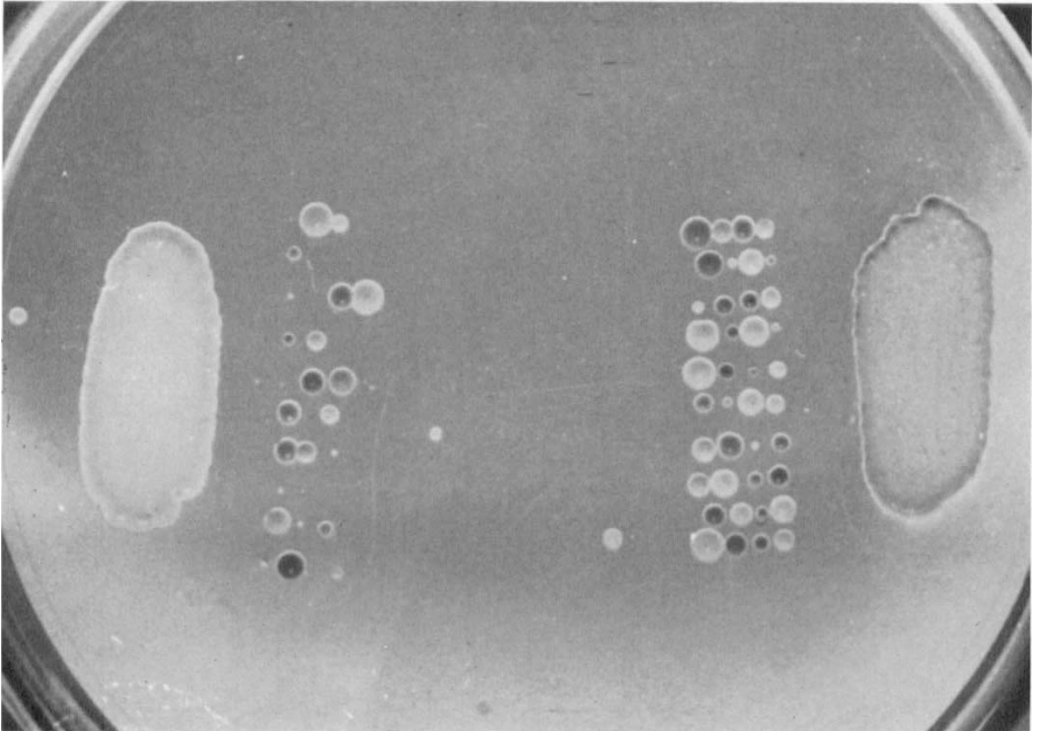
The effects of the ψ factor on the expression of the lethal phenotype. The viable S_{Q_2} haploid strain 249/5b is crossed with ψ^+ and ψ^- variants of strain 170/2c. Ten tetrads from each diploid have been dissected out. The ψ^+ diploid, 272, is on the left, the ψ^- diploid, 273, on the right (table 4, line 10 and 11).

2. A genetic analysis has been carried out which demonstrates that it is the suppressor phenotype itself which leads to the death of haploid cells carrying the mutation.

3. The possibilities that the lethal phenotype is due to a coincident, closely linked mutation, or that both the suppressing and the lethal phenotypes are the result of a deletion affecting two loci have been considered.

4. They have been ruled out by showing that various independently isolated alleles of the suppressor (including, in these strains, the super-suppressor S_D, isolated by Hawthorne and Mortimer (1963)) are also recessive lethals, and that a number of independent reversions of the suppressor from ochre-specific to amber-specific suppression were accompanied by a loss of the lethal phenotype.

5. This interpretation is reinforced by the observation that the lethal phenotype can be lost without affecting the suppressor phenotype as a result of a modifying mutation.

6. The modifier is a cytoplasmically inherited determinent, ψ. The suppressor S_{Q_2} is lethal in haploid ψ^+ strains, but not so in ψ^- strains. The determinant has previously been identified by the fact that it modifies the activity of another ochre-specific super-suppressor, S_{Q_5}, though in that case, it is the suppressing phenotype which is altered; in ψ^- strains ochre mutations are not suppressed by S_{Q_5}, while they are in ψ^+ strains.

7. The interpretation of these results takes into account certain other effects of the ψ determinant on ochre-specific super-suppressors. It is that all the effects, lethality and suppressor activity, can be related to the efficiency of suppression, that is the frequency with which ochre codons are misread and chain termination prevented. When this efficiency is too great, normal protein synthesis is seriously affected, and lethality results. When it is too small, no suppression is observed.

8. The phenomena reported would result if the rate of ochre-misreading were higher in ψ^+ strains than in ψ^- strains.

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