EXTRACHROMOSOMAL ELEMENTS IN A SUPER-SUPPRESSION SYSTEM OF YEAST

III. ENHANCED DETECTION OF WEAK SUPPRESSORS IN CERTAIN NON-SUPPRESSED *PSI*-STRAINS

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SUMMARY

Individual colonies of an adenine-requiring strain of the genotype *ade 2-1*, *SUPQ5*, [*psi*-] exhibit high and uniform frequencies of *ADE*+ revertants (5.1×10^{-5}). The frequencies for strains of the genotypes *ade 2-1*, *SUPQ5*+, [*psi*+], and *ade 2-1 SUPQ5*+, [*psi*-] are by contrast 1.2×10^{-7} and 0.5×10^{-7} respectively.

Mutation from [*psi*-] to [*psi*+], can occur. This suggests that the original mutation from [*psi*+], to [*psi*-] is not necessarily a physical loss of, or extensive deletion in, the extrachromosomal elements.

Although mutation from [*psi*-] to [*psi*+], occurs, the majority of *ADE*+ revertants from *ade 2-1*, *SUPQ5*, [*psi*-] strains contain other types of mutation. Crosses between most *ADE*+ revertants and *ade 2-1*, *SUPQ5*+, [*psi*-] strains yield adenine-requiring diploids and tetrad segregations of 0:4, 1:3 and 2:2 *ADE*+ : *ade*-. It is argued that most mutations give rise to weak recessive suppressors (*SUPX*) which are themselves incapable of suppressing the ochre allele *ade 2-1*, unless the allele *SUPQ5* is also present.

Tests with two suppressors, *S_θ* and *S_ε*, isolated by Dr R. A. Gilmore, which are themselves incapable of suppressing *ade 2-1*, show that they do not do so even in the presence of *SUPQ5*. Thus the set of suppressors isolated by us is a special class which can perhaps only be observed in the genetic background *ade 2-1*, *SUPQ5*, [*psi*-].

There is evidence that some of the new suppressors are capable, in the absence of *SUPQ5*, of weakly suppressing *trp 5-48* and perhaps *can 1-100*. All ochre alleles are, however, suppressed by the combination *SUPQ5*, *SUPX*.

SUPQ5 is able to suppress *trp 5-48*, *his 5-2*, *lys 1-1* and *can 1-100* in a [*psi*-] background, although it does so variably. It is incapable of suppressing *ade 2-1* in such a background. It is argued that the alteration in the [*psi*] determinant results in a lowered efficiency of suppression by *SUPQ5* rather than a changed specificity.

1. INTRODUCTION

THE activity of certain super-suppressors in *Saccharomyces cerevisiae* is modified by changes in an extrachromosomal hereditary determinant termed [*psi*] (Cox, 1965, 1971). In the case of the ochre-specific suppressor *SUPQ5*, the presence of [*psi*+], or [*psi*-] respectively allows or prevents suppression. Thus a strain of the genotype *ade 2-1*, *SUPQ5*, [*psi*+], will be prototrophic, whereas the otherwise isogenic [*psi*-] strain will be adenine-requiring owing to the failure of *SUPQ5* to suppress the ochre allele *ade 2-1*.

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Colonies of strains of the genotype *ade 2-1*, *SUPQ5*, [*psi*-] which are red-pigmented on complete medium were observed to throw off white papillae at a high frequency. It was of some interest to see if this apparently high mutant frequency was caused by reversion to prototrophy and, more specifically, by reversion from [*psi*-] to [*psi*+], since many extrachromosomal determinants show high rates of mutation (Jinks, 1963). As a comparison, revertant frequencies were studied in two other adenine-requiring strains both of which were wild type at the *SUPQ5* locus and contained the *ade 2-1* allele, but which were either [*psi*+] or [*psi*-].

While it is a simple technical procedure to select *ADE*+ revertants from adenine-requiring strains, the nature of the reversion must be uncovered by crossing the revertants to suitably marked tester strains since prototrophy may arise from mutations at any one of a number of functionally distinguishable loci *viz* back mutation at the *adenine-2* locus, mutation at the *SUPQ5* locus so that *SUPQ5* can function as a suppressor in a [*psi*-] background, mutations at other suppressor loci and finally, mutation in [*psi*] itself.

This paper deals with the spontaneous mutant frequencies of three genotypically different adenine-requiring strains and with the nature of some of the reversions to prototrophy.

2. MATERIALS AND METHODS

(i) *Strains and nomenclature*

The strains used are shown in table 1. The ochre suppressible alleles *arg 4-17 his 5-2, lys 1-1, trp 5-48* and *can 1-100* and the non-suppressible alleles *leu 1-12 met 1-1* come originally from the stocks of Dr R. K. Mortimer. *S₇* and *S₈* are suppressors kindly supplied by Dr R. A. Gilmore.

TABLE 1
Strains used in this investigation

Number	Mating type	<i>ade</i>	<i>arg</i>	<i>can</i>	<i>his</i>	<i>leu</i>	<i>lys</i>	<i>met</i>	<i>trp</i>	[<i>psi</i>]	Suppressors
151/3a	α	2-1	+	+	+	+	+	+	+	+	None
170/2c red	<i>a</i>	2-1	+	+	+	+	+	+	+	—	<i>SUPQ5</i>
172/9b red	α	2-1	+	+	+	+	+	+	+	—	<i>SUPQ5</i>
193/1a	α	2-1	+	+	+	+	+	+	+	—	<i>SUPQ5</i>
193/1b	<i>a</i>	2-1	+	+	+	+	+	+	+	—	<i>SUPQ5</i>
197/2d	α	2-1	+	+	+	+	+	+	+	—	None
403/5b	α	2-1	+	1-100	5-2	1-12	1-1	1-1	+	—	None
403/8c	<i>a</i>	2-1	+	1-100	5-2	1-12	1-1	1-1	5-48	—	None
403/15a	<i>a</i>	2-1	+	1-100	5-2	1-12	1-1	1-1	5-48	—	<i>SUPQ5</i>
403/16a	α	2-1	+	1-100	5-2	1-12	1-1	1-1	5-48	—	<i>SUPQ5</i>
1687-12B-R32	α	2-1	4-17	+	5-2	1-12	1-1	1-1	5-48	—	<i>S₇</i>
1687-12B-R42	α	2-1	4-17	+	5-2	1-12	1-1	1-1	5-48	—	<i>S₈</i>

Note: *Can 1-100* confers resistance to the arginine analogue, canavanine. When suppressed, the strain carrying the allele becomes sensitive.

The nomenclature follows the suggestions in the Yeast Genetics Supplement to Microbial Genetics Bulletin No. 31, November 1969. It should be noted that *SUPQ5* is the former *SUQ5* (Cox, 1965) and that [*psi*] was formerly ψ (Cox, 1965, 1971; Young and Cox, 1971). *SUPQ5*+ stands for the *wild type, non-suppressing* allele of the *SUPQ5* locus.

(ii) *Detection and isolation of ADE+ revertants*

The media have been described previously (Young and Cox, 1971). Suspensions of the strains to be tested were made up in saline and, after suitable dilution, were spread on complete (YC) plates. After 4 days' incubation at 28°C individual colonies were picked and resuspended and 0.1 ml aliquots were spread on minimal (YNB) plates. Suitable dilutions of each suspension were spread on YNB plates supplemented with adenine to estimate cell titre. The numbers of colonies growing on YNB were counted after 6 days' growth at 28°C, those on YNB+adenine after four. *ADE+* colonies from different cell populations were picked and grown up on YC for further analysis.

(iii) *Genetic analysis*

Crosses between revertants and suitably marked tester strains were performed by the methods described in Young and Cox (1971).

3. RESULTS

(i) *Revertant frequencies in three genotypically different adenine-requiring strains*

Strain 172/9b red is non-suppressed since it has a genotype *SUPQ5*, [*psi*-] in addition to the ochre mutation *ade 2-1*. It can be seen from table 2

TABLE 2

ADE+ revertant frequencies from colonies of three genotypically different adenine-requiring strains

Strain 172/9b red (<i>ade 2-1</i> , <i>SUPQ5</i> , [<i>psi</i> -])				Strain 151/3a (<i>ade 2-1</i> , <i>SUPQ5</i> +, [<i>psi</i> +])				Strain 197/2d (<i>ade 2-1</i> , <i>SUPQ5</i> +, [<i>psi</i> -])			
Colony	Cells plated $\times 10^{-4}$	Revertants	Frequency $\times 10^4$	Cells plated $\times 10^{-4}$	Revertants	Frequency $\times 10^7$		Cells plated $\times 10^{-4}$	Revertants	Frequency $\times 10^7$	
1	376	134	3.6	612	6	9.8*		289	0	<3.5	
2	434	142	3.3	681	0	<1.5		371	0	<2.7	
3	398	330	8.3	802.5	5	6.2*		354	0	<2.8	
4	551	190	3.4	649.5	406	625.1*		537	63	117.3*	
5	373	214	5.8	544.5	2	3.7		425	0	<2.4	
6	409	233	5.7	544.5	2	3.7		321	0	<3.1	
7	349	165	4.7	570	0	<1.8		412	1	2.4	
8	385	413	10.7	938	2	2.1		320	11	34.4*	
9	423	223	5.3	382.5	226	590.8*		318	0	<3.1	
10	552	330	6.0	570	0	<1.8		256	1	3.9	
11	490	186	3.8	921	0	<1.1		390	1281	3284.6*	
12	601	231	3.8	628.5	0	<1.6		335	0	<3.0	
13	346	153	4.4	444	1	2.3		484	5	10.3*	
14	310	136	4.4	555	2	3.6		365	0	<2.7	
15	—	—	—	427.5	1	2.3		356	0	<2.8	
Totals and means	5997	3080	5.1	9270.5	653	70.4		5533	1362	246.2	
				Adjusted mean = 1.2×10^{-7}				Adjusted mean = 0.5×10^{-7}			

Notes: 1. *Excluded from calculated mean frequency of revertants. See text for explanation.

2. The number of cells plated on YNB plates is calculated from a titre estimated by counting colonies on YNB plates supplemented with adenine.

that the *ADE+* revertant frequency, within each of the samples from the 14 colonies tested, was high and uniform, ranging from 3.3 to 10.7×10^{-5} with a mean of 5.1×10^{-5} . This high mean frequency presumably reflects a high rate of mutation. On the other hand, the revertant frequencies among the samples from colonies of 151/3a (*ade 2-1*, *SUPQ5*+, [*psi*+]) and 197/2d (*ade 2-1*, *SUPQ5*+, [*psi*-]) were extremely variable and generally much lower. The method of obtaining an "adjusted mean" revertant frequency

for each of the two latter strains was as follows. The samples with no revertants were grouped and the mean number of cells that were plated per sample was calculated. The numbers of revertants among each of the remaining samples were then adjusted as though they had arisen from this hypothetical mean cell population. Each of these adjusted numbers of revertants was taken in turn and the probability of obtaining zero revertants was calculated from a Poisson series. Probabilities of less than 0.05 served to exclude the sample from a calculation of overall revertant frequency. The justification for this procedure is that a high mutant frequency will result if mutation occurs early during the growth of the colony. The number of revertants is then not a true reflection of mutation rate but is heavily weighted by proliferation of revertant cells. The adjusted mean revertant frequencies for strain 151/3a and for 197/2d are 1.2 and 0.5×10^{-7} respectively, *i.e.* two to three orders of magnitude lower than that for strain 172/9b red.

Whatever the cause, the frequency with which *ADE*+ revertants are observed depends not on the extrachromosomal background of the strain but on the allele present at the *SUPQ5* locus. Strain 197/2d which is [*psi*-] but *SUPQ5*+, has a much lower revertant frequency than 172/9b red; indeed it is comparable to that obtained in strain 151/3a which is *SUPQ5*+ [*psi*+]. It was therefore of some interest to determine what mutational events had taken place in these revertants.

(ii) *Mutation from [psi-] to [psi+] in strain 172/9b red*

The high revertant frequency in strain 172/9b red (*ade 2-1*, *SUPQ5*, [*psi*-]) was thought at first to reflect high rates of mutation occurring in extrachromosomal determinants, in this case mutation from [*psi*-] to [*psi*+]. The only way to demonstrate this, however, is by means of a diagnostic cross since 172/9b red could mutate to give an *ADE*+ phenotype in a number of ways:

- (a) [*psi*-] → [*psi*+]
- (b) *SUPQ5* → *SUPQ5'* (a new allele at the *SUPQ5* locus, capable of suppressing *ade 2-1* in a [*psi*-] background)
- (c) *SUPX*+ → *SUPX* (a suppressor capable of suppressing *ade 2-1* in a [*psi*-] background)
- (d) *ade 2-1* → *ADE2*+
- (e) *ade 2-1* → *ade 2-x* (a new allele capable of being suppressed by *SUPQ5* in a [*psi*-] background)

Type (a) may be distinguished from the others by crossing the revertants to a strain of the genotype *ade 2-1*, *SUPQ5*, [*psi*-], *i.e.* a backcross. If a mutation of type (a) has occurred, diploids and, usually, *all* of the haploid offspring will be white and *ADE*+ (Cox, 1965; Young and Cox, 1972). Types (b) and (c) may give either white or red diploids depending on the dominance or recessiveness of the new suppressor mutation, while type (d) may be expected to give a white diploid since *ADE2*+ is dominant to *ade 2-1*. More importantly, however, all three types will yield segregations of 2 : 2 white *ADE*+ : red *ade*- at meiosis. Type (e) will also yield diploids whose phenotype is uncertain *a priori* and will give 2 : 2 segregations in tetrads.

Table 3 sets out the results from crosses between 111 *ADE*⁺ revertants and the [*psi*−] strain 170/2c red. At least one revertant had arisen from a [*psi*−] to [*psi*+] mutation since very few of the ascospores obtained from the cross were *ade*−. A similar mutation of [*psi*−] to [*psi*+] has been observed on an earlier occasion, when an *ADE*⁺ revertant of 179/9b red was crossed with 170/2c red and the diploid also gave rise to a majority of *ADE*⁺ segregants.

TABLE 3

Crosses between ADE⁺ revertants from strain 172/9b red (*ade* 2-1, SUPQ5, [*psi*−]) and strain 170/2c red (*ade* 2-1, SUPQ5, [*psi*−])

Number of revertants tested	Diploid phenotypes		Tetrad analysis segregations <i>ADE</i> ⁺ : <i>ade</i> −
	Colour	Requirement	
107	Red	Adenine	From 1 diploid clone: 11 × 2 : 2 1 × 1 : 2 Spore-clone total: 24 : 26 From 1 diploid clone: 3 × 4 : 0 1 × 3 : 1 1 × 2 : 2 Spore clone total: 28 : 5 (includes data from tetrad dissections where fewer than four ascospores germinated)
2	White	None	
2	Zygotes did not grow		
Total	111		

Note: 1. The 111 revertants were chosen from 34 different populations of 172/9b red. Some revertant clones may thus be related to others in the single populations from which they were picked.

Most revertants, however, seem to have arisen from other types of mutation since the majority of diploids were red and adenine-requiring. From these results, therefore, it would seem that the high revertant frequency is not explained by high rates of mutation in the extrachromosomal determinant [*psi*]. Nor is it explained by a high rate of mutation from *ade* 2-1 to *ADE*⁺, since in this case also the diploids from the backcross should be white and adenine-independent.

(iii) *The nature of reversion in SUPQ5 [*psi*−] strains*

To find out what the majority of revertants from SUPQ5 [*psi*−] were, a set of *ADE*⁺ revertants was obtained from two strains 193/1a and 1b, which are descendents of 172/9b red, and have a similar genotype and a similarly high frequency of reversion to adenine independence. These revertants were crossed with SUPQ5 [*psi*−] strains to check for mutations to [*psi*+] . The results shown in the right-hand columns of table 4 indicate that there were no such mutations in the revertants tested and that the reversions were recessive except for 193/1b-r6. This exception will be dealt with in Section (iv). The revertants were also crossed to SUPQ5+ [*psi*−]

TABLE 4

Data from crosses between revertants from strains 193/1a and 193/1b (ade 2-1, SUPQ5, [psi-]) and strains which are ade 2-1, SUPQ5+, [psi-] or ade 2-1, SUPQ5, [psi-]

Revertants from 193/1a	Diploid phenotype from cross to SUPQ5+, [psi-]	Number of tetrads segregating for ADE+ : ade-			Diploid phenotype from cross to SUPQ5, [psi-]	Number of tetrads segregating for ADE+ : ade-		
		0 : 4	1 : 3	2 : 2		0 : 4	1 : 3	2 : 2
r1	—	—	—	—	Red	—	—	—
r2	Red	2	3	1	Red	0	1	7
r3	—	—	—	—	Red	—	—	—
r4	—	—	—	—	Red	—	—	—
r5	Red	0	0	1	Red	—	—	—
r6	—	—	—	—	Red	—	—	—
r7	Red	No asci			Red	0	0	7
r8	Red	1	3	0	Red	—	—	—
r9	Red	4	3	0	Red	—	—	—
r10	Red	0	2	4	Red	—	—	—
r11	—	—	—	—	Red	—	—	—
Revertants from 193/1b								
r1	Red	1	2	1	Red	0	0	6
r2	Red	1	5	1	—	0	0	2
r3	Red	2	3	2	Red	—	—	—
r4	Red	1	4	4	Red	—	—	—
r5	Red	0	4	1	Red	0	0	9
r6	White	0	0	6	White	9 at 2 : 1		
r7	Red	1	8	0	Red	—	—	—
r8	Red	0	1	1	Red	1	0	4

Notes: 1. 193/1a-r5 also had four segregations at 1:2, 2 at 1:1 and 3 at 0:2.

2. The SUPQ5+, [psi-] strains were 403/5b and 403/8c.

3. The SUPQ5, [psi-] strains were 193/1a, 193/1b, 403/15a and 403/1a.

4. All revertants were chosen from 19 independent populations of 193/1a and 193/1b.

strains which carried a number of ochre alleles. The diploids may be represented as:

$\frac{ade\ 2-1}{ade\ 2-I'} \frac{\text{various ochre alleles}}{+} \frac{+}{+} \frac{+}{+}$ if mutations of type (c) have occurred

or:

$\frac{ade\ 2-1}{ade\ 2-x'} \frac{\text{various ochre alleles}}{+} \frac{+}{+}$ if mutations of type (e) have occurred

or:

$\frac{ade\ 2-1}{ade\ 2-I'} \frac{\text{various ochre alleles}}{+} \frac{+}{+}$ if mutations of type (b) have occurred.

If, in type (c), both suppressors are required for the expression of an ADE+ phenotype, digenic segregations will yield tetrads of 0:4, 1:3 and 2:2 ADE+ : ade- spore clones. Similarly, in type (e), the digenic segregation of the adenine and suppressor loci (known from previous studies to be unlinked) will yield tetrads of 0:4, 1:3 and 2:2 ADE+ : ade-. If, on the other hand, in type (c), SUPX is capable of suppressing ade 2-1, whether or not SUPQ5 is present, tetrads will segregate 2:2. Crosses involving mutations of type (b) will also segregate 2:2.

The left-hand columns of table 4 contain the results for adenine phenotypes in both diploids and haploid segregants from crosses of the general

type "193/1a (or 1b) — revertant $\times ade$ 2-1, *SUPQ5*+, [*psi*—]". The tetrad segregations reveal the revertants, except for 193/1b-r6, must contain *either* recessive suppressors of type (c) which are incapable of suppressing *ade* 2-1 except in the presence of *SUPQ5*, or *ade* 2-*x* alleles. The latter possibility is excluded by observations on the histidine, lysine, tryptophan and canavanine phenotypes of the *ADE*+ spores. Such spores, with only two exceptions, were *HIS*+, *LYS*+, *TRP*+ AND *CAN*^s (table 5 (d)) despite the fact that the suppressible mutant alleles were segregating in the crosses. We conclude that many—if not all—of the revertants contain weak recessive suppressors incapable of suppressing *ade* 2-1 except in the presence of *SUPQ5*.

TABLE 5
Pooled spore-clone data from the crosses of table 4 (left-hand columns)

Growth	Histidine	Lysine	Tryptophan	Canavanine
(a) <i>SUPQ5</i> clones (positively identified by cross-streaking to <i>ade</i> 2-1, <i>SUPQ5</i> +, [<i>psi</i> +] strains)				
+	56	47	37	19
±	20	17	0	14
—	2	14	0	45
(b) <i>SUPX</i> clones (identified by deduction from 0 : 4 <i>ADE</i> + : <i>ade</i> — tetrads)				
+	15	16	13	11
±	0	0	0	1
—	13	12	1	16
(c) <i>SUPX</i> or + clones ("not- <i>SUPQ5</i> " segregants in 1 white : 3 red tetrads: suppression will be revealed as an excess of clones scored as " + ", or "sensitive" in the case of canavanine resistance)				
+	42	38	20	36
±	1	1	0	4
—	38	42	9	41
(d) <i>SUPQ5-SUPX</i> clones (identified as white <i>ADE</i> +))				
+	71	72	26	1
±	1	1	1	6
—	1	0	1	66
(e) Clones containing neither suppressor (identified by deduction from 2 : 2 <i>Ade</i> + : <i>ade</i> — tetrads)				
+	15	14	4	18
±	0	0	0	0
—	18	19	6	15

Notes: 1. Failure to grow on canavanine-containing media implies suppression of the *can* 1-100 resistance allele.

2. ± This is a subjective estimate of limited growth on the particular omission medium.

3. 5(e) serves as a control on the Mendelian segregation of the ochre alleles. The non-suppressible *leu* 1-12 and *met* 1-1 also segregated normally (2 : 2) in tetrads (*leu* 1-12: 69 $\times$ 2+ : 2—, 1 $\times$ 1+ : 3—; *met* 1-1: 68 $\times$ 2 : 2, 1 $\times$ 3 : 1, 1 $\times$ 1 : 3).

It is of course possible that the suppressed spores among these segregants are occasional [*psi*+] strains. Red, *ade*— segregants from these crosses were in turn crossed with *SUPQ5* [*psi*—] strains. No *ADE*+, white diploids were ever formed suggesting that the [*psi*] factor was not segregating. Furthermore, in crosses where *SUPQ5* was homozygous (right-hand column of table 4) the segregation of suppressed : non-suppressed phenotypes was 2 : 2; not, in our experience, characteristic of the behaviour of the *psi* factor.

It is known that super-suppressors in yeast may be grouped according to the pattern of suppressible alleles which they will suppress (Magni and Puglisi, 1966; Gilmore, 1967; Hawthorne and Mortimer, 1968). Some of the revertants isolated in this study might contain suppressors which, although not capable of suppressing *ade 2-1* by themselves, might suppress other ochre alleles. Furthermore, it is of some interest to see whether or not *SUPQ5* can suppress some of these alleles in a [*psi*-] background. The tetrad data from the crosses on the left-hand side of table 4 were pooled to examine these two problems. White spore clones contain two suppressors, *SUPQ5* and *SUPX*, while red spore clones may contain *SUPQ5*, *SUPX*, or neither. These may be distinguished by cross-streaking with an *ade 2-1*, [*psi*+] strain since *SUPQ5* cultures will yield diploids which are white *ADE*+ owing to the fact that *SUPQ5* is a dominant suppressor of *ade 2-1* in a [*psi*+] background. Those cultures, which do not yield white diploids on cross-streaking, may contain *SUPX* or no suppressor. In tetrads segregating 0 : 4 and 2 : 2 *ADE*+ : *ade*- this ambiguity may be resolved but not in 1 : 3 tetrads.

In table 5 three phenotypes have been recorded for each trait segregating: + (good growth on the tester medium), $\pm$ (very weak growth) or - (none). The segregants have been grouped according to the suppressors each contains. Within each group of segregants ((*a*)-(*e*) except grouping (*c*)) a wholly non-suppressed, allele should segregate in a ratio of 1+ : 1-. If it is consistently suppressed, all segregants should be "+" and if suppression occurred only sporadically, there should be an excess of "+" segregants. In interpreting the results, we may take ambiguous scores ($\pm$) as positive indicators of suppression.

Several points become clear:

(i) Grouping (*a*) shows us that *SUPQ5* is a variable suppressor of *his 5-2*, *lys 1-1* and *can 1-100* and a consistent suppressor of *trp-5.48* in a [*psi*-] background. The excess of "+" over "-" scores for growth without histidine or lysine (or the reverse with canavanine) is significant and there are several ambiguous scores for each of these phenotypes.

(ii) From (*b*) we see that, overall, the various *SUPX* mutations only suppress *trp 5-48*. There is no evidence for suppression of *can 1-100*, *his 5-2* or *lys 1-1*. These conclusions are supported by the data in grouping (*c*).

(iii) Grouping (*d*) shows that, with few exceptions, the combination *SUPQ5*, *SUPX* suppresses all the ochre alleles.

(iv) *The nature of the revertant 193/1b-r6*

193/1b-r6 was the one exception to the rule that revertants from 193/1a or 1b gave red diploids on crossing to *ade 2-1* [*psi*-] strains which were either *SUPQ5* or *SUPQ5*+. This exception gave white diploids in both types of cross and the tetrad segregation was 2 : 2, *ADE*+ : *ade*-. Where other ochre alleles were segregating the *ADE*+ spores were prototrophic for the requirements in question. This revertant must therefore contain a dominant suppressor capable of suppressing ochre alleles in a [*psi*-] background. The type of suppressor is uncertain, however, because it could be a new allele of *SUPQ5*, a new suppressor capable of acting independently of *SUPQ5* or a new suppressor which can only act jointly with *SUPQ5*, but

which is closely linked to it so that few 0 : 4 or 1 : 3 *ADE* + : *ade* - segregations occur in the cross to a *SUPQ5* + *SUPX* + strain. None of the red (*ade* -) segregants from the cross with a *SUPQ* + [*psi* -] strain contained *SUPQ5* or were *psi* +.

(v) *Other crosses involving known suppressors*

To see if it is a general rule that suppressors incapable of suppressing *ade 2-1* can do so if coupled with *SUPQ5*, crosses were performed between strains containing either of the Class III set 1 suppressors, *S_θ* and *S_ζ* and *SUPQ5* [*psi* -] strains. The diploids would be of the following type:

$$\frac{\text{ade 2-1 } SUPQ5}{\text{ade 2-1'}} \frac{+}{+} \frac{[psi-]}{S_\theta \text{ or } S_\zeta}$$

If *SUPQ5* and *S_θ* or *S_ζ* can combine to provide a suppressed phenotype in a haploid spore this will be revealed as a 2 : 2 or 1 : 3 segregation. In seven tetrads from each cross, all tetrads revealed a 0 : 4 *ADE* + : *ade* - segregation even though *SUPQ5* and *S_θ*, or *SUPQ5* and *S_ζ* can reassort freely at meiosis. This implies that spores which contain both *SUPQ5* and *S_θ* or both *SUPQ5* and *S_ζ* are *not* suppressed in a [*psi* -] background.

4. DISCUSSION

The initial observation that adenine-requiring strains of the genotype *ade 2-1*, *SUPQ5* [*psi* -] threw off white papillae at a high frequency led us to suspect that we had discovered another example of high rates of mutation in extrachromosomal determinants, in this case mutation from [*psi* -] to [*psi* +]. That this was not so was only revealed after a series of crosses between revertants and strains of the parental genotype. These crosses revealed that mutation from [*psi* -] to [*psi* +] can occur but is not the major component in the high reversion frequency. The fact that high frequencies of revertants were only found in strains which contained both the *SUPQ5* allele and [*psi* -] suggested that the new mutations were giving rise to weak suppressors which were only capable of suppressing *ade 2-1* in the presence of *SUPQ5*. This was supported by the evidence from crosses of the type *SUPQ5* +, [*psi* -] × “*SUPQ5*, [*psi* -] - revertant” in which digenic segregations of 0 : 4, 1 : 3 and 2 : 2 *ADE* + : *ade* - were observed. The only alternative would be that a new allele, *ade 2-x*, was arising from *ade 2-1* at a high frequency and was capable of being suppressed by *SUPQ5* in a [*psi* -] background. This alternative was ruled out by the observation that almost all white *ADE* + spores were also prototrophic for other suppressible markers. If the argument is accepted that we are dealing with weak suppressors, only capable of suppressing *ade 2-1* when they are coupled with *SUPQ5*, there is evidence some suppressors tested are capable of weakly suppressing a variety of other ochre alleles. It should be pointed out that the spectrum of suppressors being picked up may well be a special class since not all suppressors which are incapable of suppressing *ade 2-1* by themselves do so when coupled with *SUPQ5*. *S_θ* and *S_ζ* are examples of this. If this is a special class of suppressor mutation, the frequency of occurrence is much higher than the generally accepted level for *all* suppressors obtained using the Hawthorne and Mortimer suite of suppressible mutants.

Frequencies of suppressor mutations of 10^{-6} to 10^{-7} have been quoted (Magni and Puglisi, 1966). In the case of suppression of specific alleles even lower frequencies are recorded. For example, von Borstel, Cain and Steinberg (1971), using the suppressible allele *lys 1-1*, give figures of 4.1×10^{-8} for the spontaneous rate of suppressor mutation, and increases of six- to eight-fold when the strains contained the radiation sensitive alleles *rad 18-1* and *xs 1-1* (*rad 52-1*). It seems therefore that the enhanced rate of mutation we observe may well be accounted for by the rather special genetic background in which we chose to look for it.

The question arises whether *SUPQ5* is a "mutator" gene. We think it more likely that, in these strains of genotype *ade 2-1 SUPQ5 [psi-]*, *SUPQ5* is only mimicking a mutator gene in the sense that it is exposing a large number of spontaneously-occurring suppressor mutations that occur, but are unexpressed in its absence. The high "mutation rate" observed by Bridges *et al.* (1970) in a strain of *E. coli* containing a reverted amber suppressor and by von Borstel *et al.* (1973) in certain strains of yeast may be due to a similar phenomenon.

The data presented here suggest that the mutation from *[psi+]* to *[psi-]* is not always a physical loss of the determinant or a large deletion, since back mutation from *[psi-]* to *[psi+]* has been detected. This contrasts with the behaviour of *[rho]*, the extrachromosomal determinant for respiratory phenotype which apparently never mutates back from *[rho-]* to *[rho+]* (Ephrussi, 1953).

The idea that *[psi]* affects the efficiency of suppression rather than its specificity, put forward by Cox (1971), is supported by the observation that *SUPQ5* in a *[psi-]* background, while never capable of suppressing *ade 2-1*, can suppress *trp 5-48* and, sometimes, *his 5-2*, *lys 1-1* and *can 1-100*. All these alleles are efficiently suppressed by *SUPQ5* in a *[psi+]* background. This suggests that the level of completed polypeptide chains for the *trp 5*, *his 5*, *lys 1* and *can 1* gene functions is sufficiently high in some *SUPQ5 [psi-]* strains for a pseudo-wild-type phenotype to be obtained.

It would seem that the data in table 5 give clearer indications than has occurred before in our experience that *SUPQ5* is a suppressor in its own right and not a modifier which allows the expression of a suppressor gene carried on the *psi* factor determinant.

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