SUPER-SUPPRESSORS IN *SACCHAROMYCES CEREVISIAE*[1,2]

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SUPPRESSOR mutations which have the ability to simultaneously revert several mutant phenotypes were first reported in the yeast *Saccharomyces cerevisiae* by HAWTHORNE and MORTIMER (1963). These "super-suppressors," characterized by allele specificity and locus nonspecificity, were shown to affect approximately one fourth of the ultraviolet-induced mutants in this yeast.

Instances of a single suppressor gene affecting several mutant loci have been found in many organisms and have been cited in a review of suppression by GORINI and BECKWITH (1966). The review emphasizes the results obtained with the amber mutants (EPSTEIN *et al.* 1963), ochre mutants (BRENNER and BECKWITH 1965) of bacteriophage and their suppressors.

HAWTHORNE and MORTIMER (1963) reported three super-suppressors in *Saccharomyces cerevisiae*, but two of these are allelic or very closely linked. MANNEY (1964) had described an additional super-suppressor, and MORTIMER and HAWTHORNE (1966) have reported two more such loci. Further genetic studies on super-suppressor loci in yeast are presented here.

MATERIALS AND METHODS

Yeast cultures: Heterothallic strains of *Saccharomyces cerevisiae* obtained from DR. R. K. MORTIMER were employed in these experiments. The symbols and nomenclature employed for genetic markers, other than super-suppressors, are those established at the 1961 Carbondale Yeast Genetics Conference (VON BORSTEL 1963). Symbols for the five previously reported super-suppressors are those established by HAWTHORNE and MORTIMER (1963). The following strains were used for suppressor induction: X1687–12B α tr_{5-48} ar_{4-17} hi_{5-2} ly_{1-1} ad_{2-1}; X1687–16C a tr_{5-48} ar_{4-17} hi_{5-2} ly_{1-1} ad_{2-1} le_{1-12} met_1. With the exception of le_{1-12} and met_1 all alleles were known to be suppressed by the super-suppressor S_d (MORTIMER unpublished results).

The culture media and genetic methods have been described (MANNEY 1964).

Random spore analysis was performed on some hybrid strains. The diploid strains were incubated at 30°C for 3 days on presporulation media and then replica plated onto sporulation media. The sporulation plates were incubated for 3 to 4 days, after which distilled water suspensions of the hybrids were made. Hemocytometer counts were made on each suspension to determine the titre of four-spore asci, three-spore asci, two-spore asci, and single cells (diploids which did not sporulate or one-spore asci). The suspension was then centrifuged, decanted and resuspended in 0.1 ml of a solution containing snail digestive juice (Glusulase; Endo Laboratories, New York). After 1 to 2 hours treatment, the suspension was diluted to a volume of 10 ml and sonicated for

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3 minutes to disperse the spore groups. An M.S.E. Ultrasonic Disintegrator (Cat. No. 3000) with a 1-cm diameter probe operated at 1.0 to 1.3 amperes was used. The cells were plated on YEPD (complex medium containing yeast extract, peptone, and dextrose). The dilutions and volumes plated were adjusted so that the number of viable cells per plate was in the order of 100. This treatment dispersed all spore groups so that only single spores and single cells were in the suspension. Viability of the total cells (single spores plus diploids) was approximately 25%. Diploid cells as well as single spores were able to survive this treatment. Haploid colonies were identified as those that expressed the phenotype of heterozygous recessive genes in the hybrid.

All YEPD master plates were incubated for 1 day and replica plated onto omission media. These omission plates were incubated for 7 days and the nutritional phenotypes were scored at 1, 3, 5, and 7 days.

Mutation induction: Mutations were induced in strains X1687–12B and X1687–16C by ultraviolet light (UV), using a dose of 5.37×10^4 ergs/cm^2. The strains were grown on YEPD plates for 1 day and then suspended in distilled water. Aliquots of these suspensions containing approximately 2×10^7 cells were plated on each of the following single omission media: —TR, —AR, —HI, —LY, —AD (omitting tryptophan, arginine, histidine, lysine, or adenine). One half of each plate was irradiated (about 50 to 60% survival) and the plates were incubated at 30°C for 4 days. The revertant colonies were isolated onto YEPD plates. Some of the tryptophan revertants fed the background of nonrevertant cells to produce a halo of growth. This made it difficult to ascertain which colonies were revertants and which were being fed. Thus, some of the true tryptophan revertants may have been missed. The YEPD master plates were incubated for 1 day and then each was replica plated onto media —TR, —AR, —HI, —LY, or —AD. The omission plates were incubated for 7 days and scored at intervals of 1, 3, 5, and 7 days.

RESULTS

Classification of revertants: Of 1053 UV-induced revertants tested, 83 reverted for two or more of the nutritional requirements and were considered to contain super-suppressors. Revertant colonies that were able to grow on only one of the omission media (single revertants) were not analyzed. It is quite possible that the single revertants were due to suppressors or possibly even super-suppressors which suppressed alleles other than the ones studied. The numbers of total revertants and super-suppressor-bearing revertants are presented in Table 1. The percentage of multiple revertants ranged from zero (for adenine) to 22.9 (for tryptophan). The absence of suppressor-bearing revertants selected on adenineless medium, and the ability of suppressors selected on the other media to suppress the adenine requirement, could be attributed to slow growth of the suppressed

TABLE 1

Numbers of revertants (UV-induced)

	Selection medium*					
	–TR	–AR	–HI	–LY	–AD	Total
Numbers of revertants	240	240	352	120	101	1053
Number of super-suppressor-bearing revertants	55	7	15	6	...	83
Percent total revertants bearing super suppressors	22.9	2.9	4.3	5.0	...	7.9

* About 10^7 surviving cells for each omission medium.

mutant on adenineless medium. The UV-induced revertants were isolated after 4 days incubation, whereas revertants induced with X rays (MORTIMER and GILMORE, in preparation) were isolated after 4 and 7 days incubation. Comparison of the two X-ray groups (for adenineless media) showed that those isolated at 7 days had four times as many super-suppressor-bearing revertants as the group isolated at 4 days.

The suppressor-bearing revertants can be grouped into eight classes on the basis of phenotypic expression after 7 days incubation (Table 2). Most classes can be divided into sets on the basis of the number of days of incubation prior to appearance of growth on the omission media. For example, in the class in which all five alleles are suppressed, one set of suppressors allows growth after 1 day incubation on all the omission plates, whereas a second set allows growth on four of the omission plates at 1 day, and on the fifth not until after 3 days incubation. These classifications may not all be valid, because not all were tested for their reproducibility through meiosis. The number of sets ranges from two (Classes II and VII) to seven (Class I).

TABLE 2

Phenotypic classes of revertants (UV-induced)

Class	Set	Alleles suppressed					Number revertants	Total for each class
		tr_{5-48}	ar_{4-17}	hi_{5-2}	ly_{1-1}	ad_{2-1}		
I	1	1*	1	1	1	1	26	
	2	1	1	1	1	3	5	
	3	1	3	1	1	3	1	
	4	1	3	3	3	3	2	
	5	3	1	3	3	3	1	
	6	3	3	1	3	3	5	
	7	3	3	3	1	3	1	41
II	1	1	3	3	3	.	3	
	2	1	5	5	5	.	1	4
III	1	1	3	3	.	.	16	
	2	1	3	5	.	.	3	
	3	1	5	5	.	.	1	
	4	1	5	7	.	.	1	21
IV	.	1	3	.	3	.	1	1
V	1	1	1	.	.	.	1	
	2	1	5	.	.	.	9	
	3	1	7	.	.	.	2	12
VI	.	5	.	1	.	.	1	1
VII	1	1	.	.	1	.	1	
	2	1	.	.	7	.	1	2
VIII	.	1	.	.	.	3	1	1

* Number indicates days of incubation prior to appearance of growth on the plate.

Colony morphology and suppressors: Observations on colony morphology have shown that this criterion can be correlated with the presence of a suppressor. YANOFSKY and BONNER (1955) found suppressors among the slow-growing tryptophan revertants of Neurospora. Similar results were obtained by YANOFSKY (1958) with *E. coli* and by SMITH-KEARY (1960) with *Salmonella tryphimurium.* Working with Saccharomyces, PARKS and DOUGLAS (1957) found that suppressors were associated with the nonfeeder tryptophan (tr_1) revertants in contrast to feeder colonies that were back mutants. MORTIMER, BRUSTAD, and CORMACK (1965) also obtained evidence that the nonfeeder tr_1 revertants were due to suppressors.

Table 3 presents the frequency of super-suppressor-bearing revertants as a function of revertant colony size and feeder activity. The sample analyzed involved approximately one-half the total revertants, and the feeder colonies were found only on the —TR plates. The frequencies of super-suppressor-bearing revertants are not statistically different for the different colony classes except for the small feeders, for which the sample was not large enough for valid comparison. The difference between "large" and "small" clones was purely arbitrary in assignment, but was consistent throughout the experiments. The data show that the probability that a clone bears a super-suppressor is independent of clone size and of whether or not the clone is a feeder.

Genetic analysis of suppressors: Table 4 presents the data from tetrad and random spore analysis for the suppressor × suppressor hybrids obtained from the revertants of Class I, Set 1. The segregation patterns for the individual suppressible alleles are not presented. In each case, if the scoring for one of the suppressible alleles was phenotypically plus (able to grow on omission media), the phenotypic scoring for all the suppressible alleles was plus. Similarly, if the scoring for one of the suppressible alleles was phenotypically minus (not able to grow on the omission medium), the phenotypic scoring for all the suppressible alleles was minus. In some cases, it was not possible to score the nonsuppressible allele even though the suppressible alleles were scored. This resulted from a very limited growth on the master plate and the lack of transfer of cells to the —LE plate in the process of replica plating. All of these cases were the result of two suppressors segregating in one haploid spore. This phenomenon will be discussed later. Approximately 95% of all the four-spore asci were 2:2 (growth:nongrowth) for the le_{1-12} allele (nonsuppressible). The 5% that did not show a 2:2 segregation

TABLE 3

Comparison of revertants with clone size and feeder colonies

	Feeders*		Nonfeeders	
	Large	Small	Large	Small
Number of revertants	27	7	84	449
Number of super-suppressor-bearing revertants	3	.	12	59
Percent revertants bearing super-suppressors	11.1	.	14.3	13.1

* Feeder colonies found only on the –TR plate.

TABLE 4

Spore progeny analyses of suppressor × suppressor hybrids (Class I, Set 1)

Revertants from strain X1687-12B	Revertants for strains X1687-16C									
	R1	R2	R4	R6	R7	R8	R10	R11	R12	R36
R1	31:16* / 23:23 / T	24:17 / 17:24 / T	31:7 / 22:16 / T	15:11 / 15:10 / T		23:7 / 16:14 / T	131:35 / 78:85 / T		154:0 / 83:68 / T	
R3	83:0 / 42:41 / T	31:11 / 20:17 / T		29:6 / 18:14 / T	33:0 / 14:19 / R	24:8 / 14:9 / T	26:8 / 18:12 / T	20:7 / 13:12 / T	18:12 / 13:11 / T	321:0 / 114:207 / T,R
R4	13:5 / 9:9 / T	131:0 / 64:66 / T	23:10 / 18:13 / T		T	13:12 / 13:6 / T,R	49:23 / 34:35 / T	23:12 / 17:13 / T	22:9 / 13:18 / T	29:8 / 17:14 / T
R8	22:12 / 19:8 / T			53:39 / 53:39 / R		57:18 / 24:51 / R	30:13 / 15:28 / R	56:29 / 40:45 / R	25:16 / 17:10 / T	
R9	25:11 / 16:12 / T	192:0 / 95:97 / T		215:0 / 103:97 / T,R	24:12 / 16:19 / T					
R18	13:11 / 10:12 / T	20:11 / 15:13 / T	22:11 / 18:13 / T	25:9 / 18:13 / T	20:8 / 10:18 / R	25:9 / 17:15 / T	26:8 / 17:12 / T	20:9 / 14:13 / T	47:14 / 31:30 / R	
R19	24:11 / 16:16 / T	13:13 / 8:6 / T	26:12 / 20:12 / T	25:13 / 19:12 / T	20:7 / 7:20 / R	28:9 / 19:13 / T	26:12 / 18:15 / T	252:0 / 114:137 / T,R	29:12 / 20:17 / T	
R21	25:5 / 15:15 / T	20:5 / 11:12 / T	225:59 / 135:130 / T,R	26:12 / 23:13 / T	34:9 / 21:18 / T,R	286:0 / 132:154 / T,R	30:11 / 19:16 / T	33:12 / 20:19 / T	29:10 / 18:19 / T	
R24	20:5 / 7:14 / T		32:9 / 21:17 / T				155:0 / 76:75 / T		147:57 / 103:98 / T,R	
R25	159:0 / 79:80 / T		225:0 / 110:113 / T							
R26	20:9 / 16:12 / T	19:13 / 16:16 / T	27:11 / 15:19 / T	25:12 / 18:17 / T	10:6 / 11:5 / R	25:11 / 20:15 / T	141:47 / 91:87 / T	29:11 / 18:17 / T	261:0 / 123:138 / T,R	
R27		163:0 / 86:77 / T								
R28	29:10 / 6:20 / T	24:7 / 15:14 / T		53:27 / 36:44 / T,R	17:8 / 11:12 / T	25:9 / 11:19 / T	265:0 / 123:141 / T,R	34:10 / 21:21 / T	27:9 / 18:18 / T	

* Ratios: $\frac{\text{Growth: no-growth (suppressible alleles)}}{\text{Growth: no-growth (nonsuppressible alleles)}}$.

T: Tetrad analysis. R: Random spore analysis.

resulted from gene conversion of the le_{1-12} allele (GILMORE 1966) or false tetrads or both. The tetrad analysis of hybrids between two suppressor-bearing revertants, to determine if the suppressors were at different loci, was performed on 8 to 12 asci. If spores were formed which did not contain a suppressor, it was assumed that the two suppressors represented different loci. If all the viable spores were found to carry a suppressor, a repeat was indicated. For those cases in which a repeat was indicated and for those in which possible linkage was indicated, additional asci were dissected and analyzed.

Although the data in Table 4 did not show any recombinant spores for the asci from hybrids indicated to be the same, it is possible that the two suppressors in each of these hybrids were not identical but instead were very closely linked. If only the four-spore asci are considered and the number of asci for the several hybrids for each apparent repeat are added, the maximum map distances (PERKINS 1949) if two loci are involved range from 1.44 cM (centimorgans) (S_n/S_n) to 0.42 cM (S_l/S_l). Most linkages in yeast are in excess of 3 cM; however, there are a few examples of two or three genes mapping at the same locus (MORTIMER and HAWTHORNE 1966). These may be the result of a single gene conferring two mutant phenotypes by virtue of its position in a metabolic path or may represent operons (JACOB and MONOD 1961). The size of some genes in Saccharomyces is in the order of 1 to 5 cM (JONES 1964). From this, it can be inferred that each apparent repeat most probably involves only one locus.

Table 5 presents the number of independent mutations observed for each of the suppressor loci identified in the Class I, Set 1, revertants. A minimum of seven suppressor loci was identified among 22 independent mutations. Of the four mutations not assigned to one of these seven loci, two had reverted to a nonsuppressor state, one autodiploidized, and one was not tested completely. The suppressor gene in this latter revertant was shown to recombine with S_k, S_l, S_m, S_n, S_o, and S_p, and hence is not one of these six. Further tests with this mutant in hybrids with S_q are in progress.

Tables 6 and 7 present the results of genetic analyses on the revertants from Set 1 of Class III. The 14 revertants tested were found to contain suppressors at five distinct loci. Of the remaining two mutants in this class, one had reverted to nonsuppressor and one has not been tested.

Two-suppressor effect: In analysis of hybrids formed by crossing two suppressor-bearing revertants ($S_1 + \times + S_2$), the three ascus-type segregations are expressed phenotypically by the ratio of growth:nongrowth on the omission media corresponding to the homozygous suppressible alleles. Parental ditype (PD) asci

TABLE 5

Mutational repeats for suppressor genes of the Class I, Set 1, revertants

Locus:	S_k	S_l	S_m	S_n	S_o	S_p	S_q
Number of times appeared as independent mutations:	6	5	3	2	2	3	1

TABLE 6

Spore progeny analyses of suppressor × suppressor hybrids (Class III, Set 1)

Revertants from X1687-12B	Revertants from X1687-16C							
	R22	R25	R26	R27	R28	R32	R34	R38
R30	25:9 15:19 T	21:10 17:14 T		27:7 18:16 T	28:8 19:17 T	119:0 57:62 T	17:10 13:14 T	10:9 5:14 T
R32	20:7 13:14 T	31:10 22:19 T	141:0 67:74 T	28:9 18:19 T	25:6 15:16 T	175:0 85:90 T		17:14 16:15 T
R36	23:11 13:21 T	25:17 16:26 R		36:15 23:28 R	16:8 12:12 T	28:12 20:20 T	31:7 13:25 R	
R38	24:10 18:16 T	29:12 21:20 T	35:0 20:15 T	25:12 19:18 T	30:11 22:19 T	157:0 78:79 T	29:6 17:18 T	33:8 22:19 T
R41	24:8 15:17 T	29:8 17:20 T	37:0 17:20 T	28:10 19:19 T	25:7 16:16 T	166:0 83:83 T	14:3 9:8 T	
R42	18:7 9:16 T	19:10 12:15 T	15:15 17:13 T		18:7 11:12 T	18:11 15:13 T	24:7 14:16 T	143:0 72:71 T
16C*								
R22		14:0 7:7 R		5:6 6:5 R	14:2 6:10 R		35:0 0:35† R	
R28		18:23 15:26 R		16:0 0:16† R				
R34		42:0 0:42† R		28:16 6:33 R	39:33 16:56 R			

* Outcrossed to X1687-12B to get opposite mating type.
† Only one nonsuppressible allele segregating; hence all designated as spore colonies were auxotrophic for the nonsuppressible allele.
See Table 4 for explanation of symbols.

(S_1+, S_1+, $+S_2$, $+S_2$) segregate 4:0, nonparental ditype (NPD) asci (S_1S_2, S_1S_2, $++$, $++$) segregate 2:2, and tetratype (T) asci (S_1+, S_1S_2, $+S_2$, $++$) segregate 3:1. A relation was consistently observed between the distribution of spore-colony sizes and type of ascus. Parental ditype asci always were associated with four

TABLE 7

Mutational repeats for suppressor genes of the Class III, Set 1, revertants

Locus:	S_s	S_t	S_u	S_v	S_w
Number of times appeared as independent mutations:	6	2	1	3	2

NUMBER OF SUPPRESSORS SEGREGATING	ASCUS TYPE	RELATIVE SPORE COLONY SIZE (ON STOCK MAINTENANCE MEDIA)	SCORING ON OMISSION MEDIA (+ ABLE TO GROW; - UNABLE TO GROW)	SUPPRESSOR GENOTYPE
NONE	PARENTAL DITYPE	○	-	++
		○	-	++
		○	-	++
		○	-	++
TWO	PARENTAL DITYPE	○	+	$\frac{S_1}{-}$•
		○	+	$\frac{S_1}{-}$+
		○	+	+$\frac{S_2}{-}$
		○	•	+$\frac{S_2}{-}$
TWO	NONPARENTAL DITYPE	○	-	++
		○	-	++
		o	+	$\frac{S_1}{-}\frac{S_2}{-}$
		o	+	$\frac{S_1}{-}\frac{S_2}{-}$
TWO	TETRATYPE	○	+	$\frac{S_1}{-}$+
		○	-	++
		○	+	+$\frac{S_2}{-}$
		o	+	$\frac{S_1}{-}\frac{S_2}{-}$

FIGURE 1.—Relative spore colony size and phenotype. The right-most column is based on the proposition that two suppressors in the same cell retard growth.

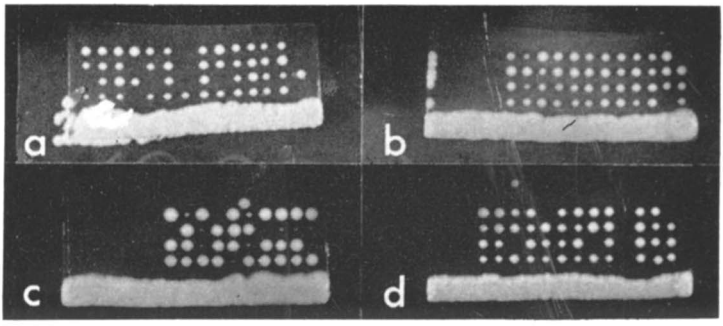

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FIGURE 2.—Spore colony growth of tetrads from crosses involving different suppressor combinations. The four spores from individual asci are in vertical columns. The medium consists of yeast extract, peptone, dextrose (stock maintenance medium) .(a) No suppressor segregating. When scored on omission media (—TR, —AR, —HI, —LY, or —AD), none were able to grow. (b) Diploid homozygous for one suppressor. All able to grow on omission media. (c) Diploid heterozygous for two different suppressors. The scoring of each spore isolate on omission media is given (+ able to grow; — not able to grow). (d) Diploid heterozygous for one suppressor. The scoring on omission media is 2:2 (+:—) for each ascus.

spore-colonies of the same diameter, NPD asci had two normal-diameter spore-colonies (the same diameter as the spore-colonies in the PD asci) and two colonies of greatly reduced diameter, whereas tetratype asci had three normal and one small-spore colony. Figure 1 is a diagram of this observed effect. In Figure 2, photographs of spore colonies from hybrids expected to express or not express this effect are presented. When no suppressor, one suppressor or two identical suppressors were in the cross, all the spore colonies were normal size. Only when two different suppressors were segregating was the above pattern observed (Figure 2C). The slow-growing colonies in the NPD and T asci were always phenotypically positive and the results were consistent with the hypothesis that slow growth was related to the presence of two suppressors in the same cell.

To test this hypothesis further, many small-spore colonies from NPD and T asci were mated to either X1687-12B or X1687-16C, the resulting diploids were sporulated, asci dissected, and the spore isolates scored phenotypically. Because there were no suppressors in either X1687-12B or X1687-16C, the presence of one or two suppressors brought in from the small-spore colony could easily be detected. If only one suppressor were present in the diploid, each ascus would have a 2:2 segregation of growth:nongrowth on omission media. If two suppressors were brought in from the small-spore colony, there would be an assortment of the

TABLE 8

Tetrad analyses of spore isolates suspected of carrying two suppressors

Suspected spore isolate	Outcrossed parent (no suppressor)	Segregations (4-spore asci)						
		Suppressible			Nonsuppressible			
		4:0	3:1	2:2	4:0	3:1	2:2	1:3
XG200–12A	X1687–16C	1	2	.		*		
XG201–3B	X1687–16C	2	2	1	.	.	5	.
XG202–4C	X1687–16C	2	3	1	.	1	5	.
XG202–5B	X1687–16C	2	2	3	.	.	6	1
XG204–2D	X1687–12B	2	3	2	.	.	7	.
XG204–3A	X1687–12B	2	1	3		*		
XG204–5B	X1687–12B	1	4	.		*		
XG205–2B	X1687–16C	2	5	1		*		
XG205–5C	X1687–16C	1	3	1	.	.	4	.
XG207–4C	X1687–12B	1	2	.	.	.	1	.
XG212–4C	X1687–12B	1	3	2	.	.	6	.
XG212–7C	X1687–12B	1	7	1	.	.	9	.
XG218–5C	X1687–16C	1	7	1		*		
XG220–1B	X1687–12B	3	4	1		*		
XG220–4B	X1687–12B	1	6	1		*		
XG220–5B	X1687–12B	1	4	1		*		
XG220–6A	X1687–16C	.	3	.		*		
XG222–6A	X1687–16C	1	3	1	.	.	5	.
XG224–2B	X1687–12B	1	5	1	.	1	4	.
XG224–7D	X1687–12B	1	.	2		*		
XG224–8C	X1687–12B	1	5	2		*		

* No segregation. Both parents carried the same allele.

suppressors among the spore progeny resulting in 4:0, 3:1, and 2:2 segregations for growth:nongrowth on omission media. Table 8 presents the results of the experiments on the spore colonies suspected of bearing two suppressors. All spores suspected of bearing two suppressors on the basis of segregation ratios and spore colony size were confirmed to contain two suppressors. In addition, the same pattern of spore colony size and phenotypic scoring was evident in the spore isolates of these hybrids. *Thus, having two suppressors in one haploid cell is deleterious in that it retards growth on YEPD medium.*

The diameters of a number of spore colonies known to contain no, one, or two suppressor genes were determined, using an American Optical Company micrometer ocular on a Bausch and Lomb dissection microscope. The measurements are presented in Table 9. These data show that each type of spore has a distribution of colony diameters. They also show that the presence of one suppressor in a haploid spore does not affect the spore-colony diameter. The most striking effect is the fivefold difference in colony diameters between spores with no or one suppressor and spores with two suppressors.

An additional line of evidence bearing on the effect of two suppressor genes involves the comparison of individual cells of the various types. Figure 3 presents photomicrographs of four different strains, two not bearing suppressors (3a,b) and two bearing one suppressor each (3c,d). Individual cells in these four pictures are all approximately the same size. Figure 4 presents photomicrographs of clones formed from single haploid cells after 24 hours incubation. Figure 4a is a photograph of a clone formed from a cell bearing one suppressor, whereas Figures 4b to 4d show clones formed from haploid cells bearing two suppressors. The clones formed by cells with two suppressor genes are much smaller than those formed by a cell bearing one suppressor. Also, the cells with two suppressors are much larger than the cells bearing either one suppressor or no suppressor. The swollen appearance is suggestive of the unbalanced growth of certain auxotrophs on minimal medium, or of progeny of irradiated cells. Thus, there are definite morphological differences associated with the presence of two suppressors in a haploid cell.

TABLE 9

Spore-colony diameter

Spore genotype	Number of spore colonies	Spore-colony diameter†	
		Average diameter	σ
No suppressors; from XG99 with no suppressors segregating*	20	141.8	22.4
One suppressor; from hybrids with two suppressors segregating	100	142.1	42.2
No suppressor; from hybrids with two suppressors segregating	45	155.0	43.8
Two suppressors; from hybrids with two suppressors segregating	36	29.3	18.7

* XG99 is the hybrid of X1687-12B crossed with X1687-16C.
† Given in micrometer units. 1 micrometer unit $= 9.25 \times 10^{-3}$ mm.

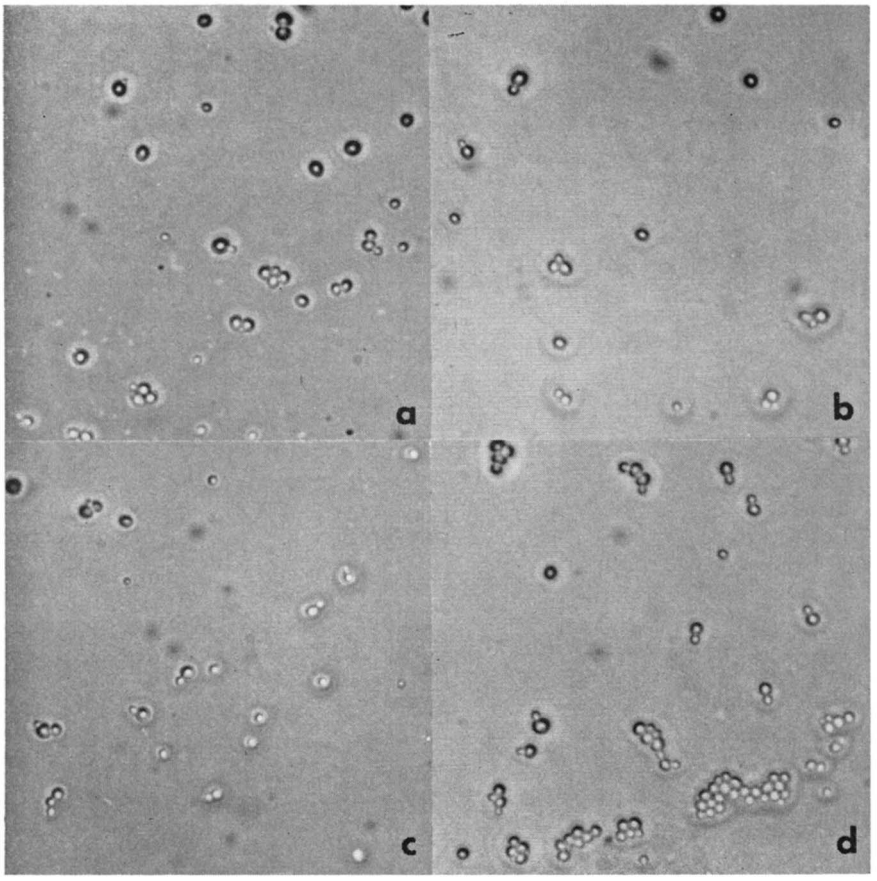

FIGURE 3.—Photomicrographs of single cells grown on stock maintenance medium (420×). (a) No suppressor, α mating type. (b) No suppressor, a mating type. (c) One suppressor, α mating type. (d) One suppressor, a mating type.

Although the "two-suppressor" effect was evident in all hybrids of Set 1 of Class I that were analyzed by tetrad analysis, there were some asci which were not consistent with this pattern. For example, some asci had four spore-colonies of normal size and yet segregated 3:1 for growth:nongrowth, and there were some PD asci with one small spore-colony. Of the total number of asci analyzed, 94.2% were in agreement with the hypothesis of the two-suppressor effect. Most of the 91 exceptional asci can be accounted for by one of three possible explanations (Table 10): (1) the spore tetrad isolated was not from a single ascus, i.e., false tetrad; (2) small spore-colonies may be due to segregational or cytoplasmic petites; (3) one of the suppressor genes failed to segregate in a 2:2 fashion, i.e., gene conversion. These asci were grouped according to the three categories, and several asci from each category were analyzed. In all, about 40% of the exceptional asci were so analyzed. False tetrads were determined on the basis of scoring markers not affected by the suppressors. If these markers (mating type and le_{1-12})

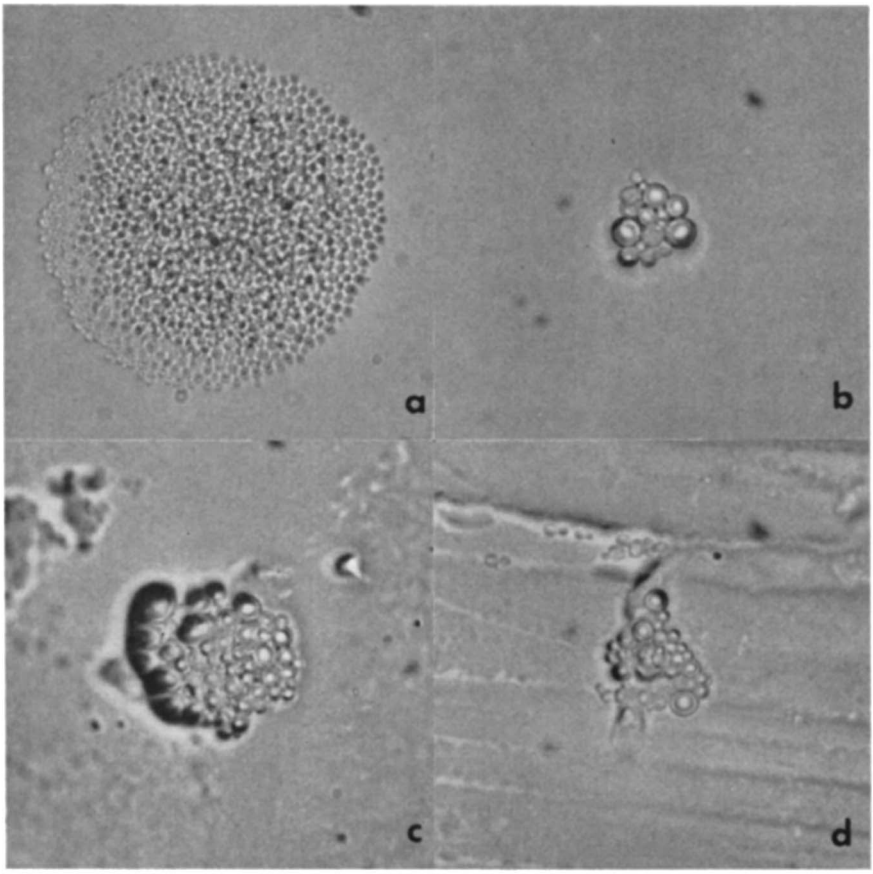

FIGURE 4.—Photomicrographs of clones derived from single haploid cells after 24 hours incubation on stock maintenance medium (420×). (a) One suppressor. (b) to (d) Two suppressors.

segregated 2:2, it was assumed that a true tetrad had been isolated. The presence of spontaneous petites as an explanation of slow growth was scored by replica plating the master plates on petite medium. Gene conversion of one of the suppressors (i.e., 1:3 segregation) was assayed by first determining that the asci were not false tetrads and then outcrossing each spore in the ascus to either X1687-12B or X1687-16C. These diploids were sporulated, asci dissected, and the spore isolates scored. If each suppressor-bearing spore carried only one suppressor, the spore

TABLE 10

Spore colony growth patterns

Number of hybrids	Number of asci	Number of asci showing two-suppressor effect	Number of exceptional asci	Number of exceptional asci that are due to:			
				False tetrads	Petite	Gene conversion	Other
85	1558	1467	91	23	47	14	7

isolate scoring would be 2:2 (+:−). Only 2:2 asci were obtained in such crosses. Some asci could not be adequately classified. It is possible that these are either false tetrads which, by chance, gave 2:2 segregation for the nonsuppressed markers or some such combination as a false tetrad and gene conversion.

Is the two-suppressor effect a characteristic of all super-suppressors? KAKAR (1963) reported what appeared to be a two-suppressor effect while working with suppressors of the isoleucine mutations in Saccharomyces. These suppressors were not shown to be multiple-site suppressors and he did not find this effect in all suppressor crosses. Examination of the spore clones from hybrids involving the suppressors from Set 1 of Class III showed that the two-suppressor effect did not occur in this case. In addition, hybrids formed by interclass crosses did not exhibit any evidence for this effect.

In summary, all pairwise combinations of two different suppressors from Class I, Set 1, exhibit the two-suppressor effect. The effect is reproduced when a haploid spore containing two suppressors is outcrossed to a nonsuppressor-bearing strain. The colonies produced by spores with two suppressors are approximately one-fifth the diameter of clones formed by spores with no or one suppressor. The cells with two suppressors are morphologically aberrant compared with cells having one or no suppressor. The effect has not been observed in spore isolates derived from two-suppressor hybrids involving other intraset or interclass and interset crosses.

Linkage: The tetrad data were examined for centromere linkage using the methods described by HAWTHORNE and MORTIMER (1960). Based on a second division segregation frequency of 46%, S_l and S_p are linked to separate centromeres. Since these two loci have not been extensively tested for linkage against genes already mapped, it is not known if these correspond to previously described centromeres (MORTIMER and HAWTHORNE 1966). Studies are under way to locate the positions of all the suppressors on the linkage map.

Interclass crosses: To determine if allelic suppressors could have different suppression patterns, hybrids containing suppressors from different classes were analyzed. Approximately one third of the possible interclass crosses were studied and the resultant data are presented in Table 11. Allelism was detected by analyzing the growth of spore progeny on omission media. The $tr_{5\text{-}48}$ mutant is suppressed by all the suppressors reported here (see Table 3). Thus, if the two suppressors in a hybrid were allelic, all spore progeny would be able to grow on the −TR plate. If the two suppressors were not allelic, recombination would result in some spore progeny not able to grow on −TR. In all cases of interclass (and interset) crosses analyzed, allelism was not detected and no interaction effect was observed. Thus it appears that each class may represent a distinct group of loci, and it may be that each set represents a unique array of loci.

Attempts to estimate the number of suppressor loci: To derive an estimate for the minimum number of suppressor loci, six of the loci from Class I, Set 1 (S_k, S_l, S_m, S_n, S_o, S_p), were crossed in all possible combinations, with two loci from Class III, Set 1 (S_s, S_t). Except for one combination, it has been shown by genetic analysis (tetrad or random spore) that each of the eight suppressors is at

TABLE 11

Interclass crosses

Parents		Class and set (respectively)		Segregation	
				Suppressible alleles	Nonsuppressible alleles
α	α	α	α	growth:nongrowth	growth:nongrowth
16C-R6	12B-R22	I-1*	I-3	29:12	21:20
16C-R1	12B-R2	I-1†	I-6	12:1	5:8
16C-R1	12B-R6	I-1†	I-6	14:3	9:8
16C-R1	12B-R7	I-1†	I-6	7:2	5:4
16C-R2	12B-R35	I-1*	III-1	18:5	9:13
16C-R17	12B-R33	II-2	II-1	32:7	20:19
16C-R16	12B-R35	II-1	III-1	10:5	10:5
16C-R16	12B-R37	II-1	III-2	5:3	5:3
16C-R16	12B-R29	II-1	III-2	9:4	6:7
16C-R16	12B-R44	II-1	III-2	9:4	7:6
16C-R14	12B-R33	III-3	II-1	23:5	14:14
16C-R15	12B-R33	III-4	II-1	21:8	16:13
16C-R5	12B-R31	VI	II-1	9:3	8:4
16C-R5	12B-R33	VI	II-1	21:5	15:11
16C-R9	12B-R31	VII-1	II-1	23:6	14:15
16C-R16	12B-R40	II-1	VII-2	21:6	16:8
16C-R17	12B-R29	II-2	III-2	16:8	14:10
16C-R17	12B-R37	II-2	III-2	34:8	21:21
16C-R14	12B-R35	III-3	III-1	19:7	14:12
16C-R15	12B-R35	III-4	III-1	17:9	17:19
16C-R5	12B-R35	VI	III-1	11:6	10:7
16C-R14	12B-R44	III-3	III-2	21:5	13:13
16C-R15	12B-R37	III-4	III-2	11:2	8:5
16C-R15	12B-R44	III-4	III-2	23:4	14:13
16C-R21	12B-R44	IV	III-2	10:13	13:10
16C-R5	12B-R44	VI	III-2	14:6	7:13
16C-R5	12B-R37	VI	III-2	23:9	16:16
16C-R5	12B-R29	VI	III-2	20:5	13:12
16C-R35	12B-R44	VIII	III-2	8:9	10:7
16C-R14	12B-R40	III-3	VII-2	15:6	9:12
16C-R15	12B-R40	III-4	VII-2	19:8	14:13
16C-R19	12B-R40	V-2	VII-2	15:2	8:9
16C-R5	12B-R40	VI	VII-2	27:5	16:16

* S_i.
† S_k.

a different locus. For this combination, $S_p \times S_t$, results are not available owing to failure to obtain a hybrid of this genotype that sporulated.

Genetic tests are being conducted to determine if the suppressors identified in this study are identical, or allelic with any of the previously identified suppressors.

DISCUSSION

Evidence cited by MAGNI and PUGLISI (1967) would indicate that the nonsense (amber and ochre) suppressors of bacteria are homologous with the super-

suppressors in yeast. Thus any model explaining the action of the bacterial suppressors might apply to the yeast suppressors. In bacteria, an altered sRNA has been shown to be involved in suppression (CAPECCHI and GUSSIN 1965). The altered sRNA could be derived from a sense-reading sRNA or from a nonsense-reading sRNA (BENZER and CHAMPE 1962). The alteration of a nonsense reading sRNA would involve only one structural gene locus. This limitation to one locus is not consistent with the data in yeast or in bacteria. The modification of a normally charged sRNA would predict a maximum of 20 classes or sets of suppressors because any one of the sRNA's could be altered to read the nonsense codon. Because there should be 62 different sRNA's which code for the 20 amino acids, there would be the possibility of many suppressor loci.

If it is assumed that the mutational event results in a change in the "anticodon" of the normally charged sRNA then the number of ways to get to a nonsense anticodon from a sense anticodon by single-step events are limited. Examining the codons, there are three types of mutational events that can convert sense to nonsense (UAG or UAA), i.e., base substitution, base addition, and base deletion. Table 12 presents a tabulation of the sense codons which can be converted to nonsense by single-step events. The corresponding amino acids are also given, as well as the mutational event that can cause the change. These 21 triplets

TABLE 12

Mutation of amino-acid code triplets from sense codons to the nonsense codon UAG

Amino-acid codon	amino acid*	Mutational event†		
		Base substitution	Base addition	Base deletion
UAU	tyr	+	+	+
UAC	tyr	+	+	+
UAA	"ochre"‡	+	+	+
UUG	leu	+	—	—
UCG	ser	+	—	—
UGG	tryp	+	+	—
CAG	gln	+	—	—
AAG	lys	+	—	—
GAG	glu	+	—	—
UUA	leu	—	—	+
AUA	met	—	—	+
CUA	leu	—	—	+
GUA	val	—	—	+
UCA	ser	—	—	+
UGA	cys or tryp	—	+	+
AGU	ser	—	+	—
AGA	arg	—	+	—
AGC	ser	—	+	—
AGG	arg	—	+	—
UCU	cys	—	+	—
UGC	cys	—	+	—

* From BRIMACOMBE *et al.* (1965) and SOLL *et al.* (1965).
† + indicates that the change to nonsense is possible by this event. — indicates that the change is not possible.
‡ Nonsense codon for ochre mutants.

represent only 11 different amino acids, and one of the triplets is the nonsense codon UAA.

This provides a maximum of 20 loci that could be converted to read nonsense but only 11 phenotypic classes would be observed (one for each of the 11 different amino acids). The number of loci per class would range from one (for four of the classes) to four (for the four serine triplets). This is consistent with the observations on *E. coli* for the numbers of suppressors, numbers of classes, numbers of loci per class, and amino-acid insertions in the presence of the suppressors.

This model does not adequately explain all the yeast results. It is consistent with the observation of 11 classes. However, the prediction of a maximum of four loci in any one class cannot account for the minimum of seven loci in Class I, Set 1. If the amino acids in Table 12 are grouped according to their side chains—e.g., acidic, basic (see STEINER 1965)—there would be a maximum of six loci for one such group. This is still not consistent with the observations in yeast.

Modification of an existing model: The model that proposes an alteration of a normal sRNA species provides an explanation for most of the observations in yeast and *E. coli* but is inadequate for some points. The data obtained in the yeast experiments reported here can lead to one modification of this model. This modification is that there exist sets of sRNA genes in which all members of a set make the same sRNA. A mutational event occurring in one of the genes of a set would be reflected in the coding specificity (for normally charged sRNA's) or in the ability to be charged (for normally uncharged sRNA's). In either case, the altered sRNA would be able to translate the nonsense codon by inserting the characteristic amino acid into the protein at that site. The remaining members of the set would function normally by translating their normal triplets. This modification obviates the necessity of a particular sRNA coding for nonsense and for its normal triplet. Because only one gene of a set is altered, the remaining members are normal and the mutational event is not lethal.

This modification makes it possible to explain all the yeast and most of the *E. coli* observations. In yeast, the existence of as many as seven distinct loci (Class I, Set 1) which are indistinguishable phenotypically could indicate that all are members of one set. This modification provides an explanation for the two-suppressor effect observed with the Class I, Set 1, suppressors. If it is assumed that all seven genes in this set code a particular sRNA, then modification of two or more of these genes to recognize nonsense might decrease to a limiting level the amount of this sRNA that is available for translating the normal triplet. Thus, the interaction effect can be explained as a reduction to a limiting level of the amount of a particular sRNA. This two-suppressor effect would not be expected in interset or interclass crosses because different sRNA sets would be involved.

For *E. coli*, this modification would explain the existence of protein fragments, as well as complete proteins, in a suppressor-bearing strain as the result of competition between the normal and mutant sRNA. The altered sRNA (whether normally charged or normally uncharged) would be competing for the nonsense codon with an uncharged chain-terminating sRNA. The efficiency of suppression

could be a reflection of the amount of gene product of the various sRNA genes, as pointed out by KAPLAN, STRETTON, and BRENNER (1965). If the various members of a set of sRNA genes were producing different quantities of sRNA, modification of the one that produced the largest amount would provide a high efficiency of suppression.

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SUMMARY

Multisite suppressors (super-suppressors) were induced in two polyauxotrophic strains of yeast. The suppressor-bearing revertants were grouped into eight phenotypic classes, and some classes were further divided into sets based on the time of expression. The presence of a suppressor in a revertant is independent of the clonal morphology. Genetic analyses established a minimum of eight super-suppressor loci. A two-suppressor effect is described which occurs when two suppressors from one class and set are combined in one haploid cell. This results in morphologically aberrant cells and retarded growth.

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