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A super-suppressor on the thirteenth linkage group in *Saccharomyces**

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1. INTRODUCTION

The phenomenon of reversion from mutant to normal or nearly normal phenotype has been proved to be due in some cases not to reversal of the original mutation, but to the suppression of its effects by the mutation of another gene (*suppressor*, Bridges, 1932), with restricted specificity in its action for the mutant allele.

A certain type of suppressor, the *super-suppressor*, is of particular interest because of its interaction with a number of nonallelic mutant loci. There are several reports of such super-suppressors in *Drosophila, Neurospora* and *Escherichia coli* (Wagner & Mitchell, 1955). Hawthorne & Mortimer (1963) have suggested three kinds of super-suppressor in *Saccharomyces*. Recently, Manney (1964) studied the mode of action of a super-suppressor on the tryptophane-synthetase gene system in *Saccharomyces*.

The present paper describes what is presumably a new super-suppressor for certain auxotrophic mutant alleles, and evidence of its possible location on the newly identified thirteenth linkage group in *Saccharomyces*.

2. MATERIALS AND METHODS

A dominant super-suppressor *S* was isolated from strain DE14, one of the standard haploid *Saccharomyces* breeding cultures for arginine marker ar_3 (we are grateful to Drs Hawthorne and Mortimer for this culture). The other cultures were selected from Dr Lindegren's yeast collection or stock cultures from Japan.

The general techniques and media for yeast genetics at Lindegren's Laboratory (Lindegren, 1962) were followed throughout in this study. Mass mating crosses were made in nutrient broth with multiple marked strains. The suppression versus non-suppression of a particular marker was recognized by the phenotypic segregation of the marker in a cross heterozygous for the suppressor, i.e., whether it gave 4+:0− or 3+:1− tetrad segregations for the particular marker besides the expected 2+:2− segregations. The regular 2+:2− segregation of other markers excluded polyploidy or extra mitosis as explanations for these aberrant segregations. The segregation of the suppressor was also examined by following the segregation of suppressed phenotypes from diploids which were homozygous for the suppressible

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marker. The suppression of several different alleles by S was confirmed by tetrad analysis of crosses in which several homozygous suppressible markers were combined with the heterozygous suppressor. In such a cross, an apparent close linkage between the suppressible markers was expected, because their phenotypic segregations were controlled by a single pair of alleles, S/S^{+}.

The procedure for mapping centromeres by tetrad analysis has been described in other studies (Shult & Lindegren, 1956; Desborough & Lindegren, 1959; Hawthorne & Mortimer, 1960; Hwang, Lindegren & Lindegren, 1963, 1964).

3. RESULTS

(i) *Specificity of suppressor* S

The results of several crosses for the detection of suppressible alleles are summarized in Table 1. It was found that suppressor S could simultaneously suppress several different auxotrophic markers (arginine-, histidine-, lysine- and methionine-

Table 1. *Specificity of super-suppressor* S

Suppressible		Unsuppressible			
ar_{3-3}	(?)	ad_{1-1}	(I)	met_{3-2}	(X)
ar_{4-1}	(VIII)	ar_{3-1}	(?)	met_{10-3}	(VI)
hi_{5-2}	(?)	ar_{3-2}	(?)	se_{1-1}	(Segt.)
ly_{1-1}	(IX)	ga_{1-1}	(II)	tr_{1-1}	(IV)
ly_{2-2}	(II)	hi_{6-1}	(IX)	tr_{3-2}	(Segt.)
met_{8-2}	(Segt.)	le_{1-1}	(VII)	tr_{5-2}	(VII)
met_{14-1}	(XI)	ly_{2-3}	(II)	ur_{3-1}	(V)
		ly_{7-1}	(XII)	va_{1-1}	(Segt.)

Symbols and terminology of genetic markers follow the suggestions of the Carbondale Yeast Genetics Conference (von Borstel, 1963).

The symbols in parenthesis show the linkage group to which the marker is assigned. *Segt.* indicates a chromosomal segment, *?* indicates a marker at an unidentified locus.

less) apparently unrelated in their chromosomal location. Thus suppressor S has multiple suppressive action, and can be called a super-suppressor. The super-suppressor, however, is highly allele-specific. For example, it shows no effects on ar_{3-1} or ar_{3-2} which are alleles of the suppressible gene ar_{3-3}. Another example of this situation was recognized at the ly_2 locus, in which ly_{2-2} is suppressed while ly_{2-3} is not.

(ii) *Dominant action of super-suppressor* S *on its wild-type allele* S+

During the course of tetrad analysis and complementation tests of sensitive markers, it became apparent that the super-suppressor was dominant to its wild-type allele S^{+}. Additional evidence was obtained by testing the phenotypes of diploid hybrids obtained from several different crosses. Each cross was hetero-

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zygous for the suppressor and homozygous for one or two of the suppressible markers. Mass mating was made in a nutrient broth, a diploid cell was isolated by means of micromanipulation, propagated vegetatively and then tested for nutritional requirements. In every case the wild-type phenotype was observed for the particular suppressible marker(s), thus supporting the view that S is dominant to S^+.

(iii) *Chromosomal location of suppressor* S

Maps of genetic markers in *Saccharomyces* have been assembled by Lindegren *et al.* (Shult & Lindegren, 1956; Desborough & Lindegren, 1959; and Hwang, Lindegren & Lindegren, 1963) and Hawthorne & Mortimer (1960). Recently, these maps were reassembled into a new chromosome map which contains twelve chromosomes, five chromosome segments carrying sites of affinity, and four chromosome segments which have not yet exhibited linkage with either a centromere or a preferentially segregating site (Hwang, Lindegren & Lindegren, 1964).

Tetrad segregations of super-suppressor S with several centromere markers on the twelve authentic linkage groups are shown in Table 2. In a cross with a suppressible marker and S (for example, $ar_{4-1}\ S^+ \times AR\ S$), *ar ar AR AR* tetrads are counted as parental ditypes (PD), *AR AR AR AR* tetrads as non-parental ditypes (NPD) and *ar AR AR AR* as tetratypes (T). In the case of a coupling cross, *AR AR AR AR* is a PD tetrad, *ar ar AR AR* is a NPD tetrad and *ar AR AR AR* is a T tetrad. The simply accumulated tetrad distributions for all the combinations between S and the centromere markers were tested by χ^2 statistics for determining the type of distribution. When

$$\chi_1^2 = \frac{(\mathrm{PD}-\mathrm{NPD})^2}{\mathrm{PD}+\mathrm{NPD}} < 6{\cdot}63$$

the difference between numbers of PD and NPD is not significant at the 1% level. When

$$\chi_2^2 = \frac{(2\mathrm{N}-3\mathrm{T})^2}{2\mathrm{N}} > 6{\cdot}63 \text{ and } 2\mathrm{N}-3\mathrm{T} > 0,$$

T is significantly less than two-thirds of the total number of tetrads (N) at the 1% level.

It is obvious that all the accumulated tetrad distributions are type F (Shult & Lindegren, 1956) distributions, except the marker ly_1 (about 35 stranes from centromere on linkage group IX) in which the value of χ_2^2 is less than the significant value 6·63 but the χ_1^2 value apparently shows no direct linkage between ly_1 and S. Another centromere marker, hi_6, which is located on the opposite side of ly_1 from the centromere on the same linkage group, shows the type F distribution with S. Therefore, S cannot be on the IXth linkage group. These data indicate that the super-suppressor S is a centromere marker of a linkage group which is not homologous to any one of the twelve previously established ones.

The map distance between S and its centromere was calculated from the accumulated tetrad distributions between ad_1 of linkage group I, mating type α of linkage

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Table 2. *Tetrad distributions exhibited by super-suppressor* S *with centromere markers of linkage groups I to XII in various crosses*

Linkage group	I	II	III	IV	V	VI	VII		VIII	IX		X	XI	XII
Marker	ad_1	ga_1	α	tr_1	ur_3	met_{10}	le_1	tr_5	ar_4	hi_6	ly_1	met_3	met_{14}	ly_7
Cross No.														
75	18 23 7	20 20 9	19 17 14		20 18 12			15 14 19*						
76			11 18 5					11 10 13						
77			13 13 8		14 10 9									
82	26 14 2		20 19 7					10 15 20						
83	15 11 3		14 11 5					12 10 7						
100			4 7 4	5 9 2	6 4 6				6 6 4		4 2 10			
101	6 6 2		11 2 4	6 8 2					5 7 5		5 5 7			
102			3 1 6		4 3 3			3 1 5		4 3 2				
106	5 8 1		6 8 1				5 9 1		9 2 4					
109			11 8 5		11 9 4	11 11 1		10 5 9						
114		4 4 1	3 3 3										3 5 1	
115		7 11 2	8 9 3										6 13 1	
118			8 5 2		7 5 4							7 5 4		
119			6 5 2		3 6 4							8 4 1		
122			10 12 6		14 9 5							16 9 3		12 6 9
123			11 9 7		9 7 11							11 10 6		13 6 5
124			7 8 7		9 9 5							10 10 2		5 4 13
125					9 11 5							13 9 4		7 7 11
126			12 7 3		7 11 4							12 8 2		11 6 5
Accumulated tetrad distribution	70 62 15	31 35 12	167 162 92	11 17 4	113 102 72	11 11 1	5 9 1	61 55 73	20 15 13	4 3 2	9 7 17	77 55 22	9 18 2	48 29 43
N	147	78	472	32	287	23	15	189	48	9	33	154	29	120
χ^2_1	0·48	0·24	0·07	1·28	0·59	0·00	1·14	0·31	0·71	0·14	0·25	3·66	3·00	4·20
χ^2_2	210·8	92·3	472·6	42·2	223·2	40·2	24·3	66·8	33·7	8·0	3·4	190·1	46·6	51·3
2N − 3T	249	120	668	52	358	43	27	159	57	12	15	242	52	111
Type of distribution	F	F	F	F	F	F	F	F	F	F	F?	F	F	F

* PD:NPD:T. (Tetrad data do not include irregular asci.)

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group III, ur_3 of linkage group V and tr_5 of linkage group VII, in which more than a hundred asci have been analyzed. The three D_{C-S} values range from 2·75 to 6·65 and their average is 4·87 or approximately 5 stranes as shown in Table 3.

Table 3. *Calculation of the distance: super-suppressor* S *to centromere*

	Tetrad distribution			Distance in stranes	
Combination	PD	NPD	T	D_2	D_{C-S}
S–ad_1	70	62	15	5·5	
S–α	70	57	31	11·7	
ad_1–α	58	68	29	6·8	5·20
S–ur_3	113	102	72	18·6	
S–α	104	92	64	15·3	
ur_3–α	102	81	94	20·6	6·65
S–tr_5	61	55	73	28·8	
S–α	78	74	42	13·1	
tr_5–α	55	49	83	36·4	2·75
				Average	4·87

$D_2 = -33{\cdot}3 \ln(1\text{-}3T/2N)$

$D_{C\text{-}S} = (D_{S\text{-}A} + D_{S\text{-}B} - D_{A\text{-}B})/2$

The average $D_{C\text{-}S}$ value was obtained from the $D_{C\text{-}S}$ values independently calculated from three different combinations of triplet markers: S-α-ad_1, S-α-ur_3 and S-α-tr_5.

4. DISCUSSION

Hawthorne & Mortimer (1963) suggested three kinds of super-suppressors (S_a, S_b, and S_c) in *Saccharomyces*. These super-suppressors have almost the same action spectrum, and showed no linkage to any centromere or any other known markers. Two of them are closely linked, and presumably belong to the same locus. Recently, Manney (1964) studied another type of centromere-linked recessive super-suppressor S_d, which is specific for some tryptophane-synthetase mutant alleles in *Saccharomyces*.

Our super-suppressor S is located on a possible thirteenth linkage group near to its centromere (about 5 stranes), which is comparable with S_d, but S has a dominant suppressive action.

Among twenty-three auxotrophic mutants so far tested, seven markers were suppressed by S. There is no evidence for linkage relationships among them or between them and S. It is difficult to imagine any biochemical function common to the enzymes controlled by these loci. However, the super-suppressor S is highly allele-specific as shown by a study of the loci ar_3 and ly_2.

The allele specificity excludes the 'metabolic balance' hypothesis (Strauss, 1960) in which the suppressor acts by introducing or removing genetic blocks in biochemically related pathways.

The work of Suskind & Kurek (1959) with a suppressor of the temperature-sensitive mutant td_{24} in *Neurospora* has shown that the suppressor appears to remove

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a normal cellular component which inhibits the activity of the mutant enzyme, but does not affect the properties of the protein itself. Campbell (1961) suggested a similar suppressing mechanism regarding mutant phages whose multiplication is more sensitive than that of the wild-type to high temperature, to extreme pH or to the presence of suppressor genes in the bacterial host.

Benzer & Champe (1961, 1962) put forward another explanation of the suppression phenomenon after genetic analysis of 'Ambivalent' *rII* mutants of phage T4, proposing the existence of coding triplets called nonsense codons which do not correspond to any amino acids. A codon which is nonsense in one bacterial host, nevertheless makes sense in another host containing a suppressor mutation. The suppressor mutation thus contributes, in a limited sense, a hereditary alteration of the genetic translating system.

After investigating alkaline-phosphatase negative mutants in *Escherichia coli*, Garen & Siddiqi (1962) also reported that the suppressor acts by converting nonsense mutations to sense. They observed that the suppressor for the alkaline-phosphatase mutants can also suppress certain *rII* mutants of phage T4.

The behaviour of super-suppressor *S* in *Saccharomyces* can most easily be explained by the nonsense model. However, there is nothing at present allowing a decision between the two models in this case.

SUMMARY

It has been proved that the suppression of seven different auxotrophic mutant alleles is brought about by a single super-suppressor allele designated *S*. Super-suppressor *S* is allele-specific rather than locus-specific and is dominant to its wild-type allele.

Suppressible mutant alleles show no linkage relationships either among themselves or with the suppressor *S*.

Super-suppressor *S* has been shown to be closely linked (about 5 stranes) to one of the centromeres other than those of linkage groups I to XII. This establishes a new thirteenth linkage group.

The possible mechanism of action of the super-suppressor is discussed.

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