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Suppressor-specificity of antisuppressors in yeast

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SUMMARY

Eighteen mutations of *Saccharomyces cerevisiae*, at eight loci, isolated as antisuppressors of *SUPQ2*, an ochre-suppressing allele of *SUP11*, were crossed with three other suppressors.

They were found to abolish the ability of *SUP2* (inserting tyrosine), to suppress the ochre mutations *ade2.1* and *can1.100*, but not its ability, to suppress *his5.2* or *lys1.1*. When coupled with any antisuppressor, *SUPQ5*, inserting serine, was also unable to suppress *ade2.1*, but the suppression of other ochre mutations varied from one *asu-SUPQ5* strain to another. No antisuppressor affected the ability of *SUP11-am*, an amber-suppressing allele of *SUP11*, to suppress *trp1.1*, an amber mutation.

1. INTRODUCTION

We have previously described the isolation and genetic analysis of a group of antisuppressor mutations in the yeast, *Saccharomyces cerevisiae* (McCready & Cox, 1973). Twenty-three such mutations were shown to map at eight loci and to affect suppression by the super-suppressor *SUPQ2*, of the ochre alleles *ade2.1*, *can1.100*, *lys1.1* and *his5.2*. *SUPQ2*, an allele of *SUP11* (Cox, 1971) is a Class I ochre-specific suppressor (Hawthorne & Mortimer, 1968). In all the mutants, suppressor efficiency appeared to be diminished rather than completely abolished. The mutations also counteracted the effect of an extra-chromosomally inherited factor, [*psi*$^+$], which has been shown to enhance the efficiency of ochre suppression (Cox, 1965, 1971).

The Class I super-suppressors, which all insert tyrosine and are ochre-specific (Gilmore, Stewart & Sherman, 1971), almost certainly code for altered tRNA species (Bruenn & Jacobson, 1972; Capecchi, Hughes & Wahl, 1975). If this is indeed the case, the phenotypic effects of the anti-suppressors could be due to any of several types of mutation:

(1) A mutant ribosomal or other component affecting codon recognition, tRNA binding, translation or polypeptide chain release.

(2) An enzyme affecting suppressor tRNA structure, for example one affecting the modification of bases or the maturation of the precursor tRNA.

(3) A mutant amino-acyl-tRNA synthetase.

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(4) An alteration in the control of tRNA synthesis or of rRNA synthesis such that the ratio of tRNA:rRNA is reduced.

A study of the specificity of the anti-suppressors was undertaken in order to begin to distinguish between these possibilities. Three suppressors were chosen: *SUP2*, like *SUP11*, a tyrosine-inserting ochre-specific suppressor (Sherman *et al.* 1973). *SUP11-am*, a specifically amber-suppressing allele of *SUP11* and *SUPQ5*, an ochre-specific suppressor inserting serine (Liebman, Stewart & Sherman, 1975). The specificities expected of the kinds of mutations outlined above are described below:

(1) An altered ribosome component affecting tRNA binding or codon recognition would probably be non-specific, except that, where recognition requires 'wobble' it would only affect the ochre-specific suppressors (with IUA, possibly, as the anti-codon). An antisuppressor affecting polypeptide chain-termination would be codon-specific rather than amino-acid-specific, if at all.

(2) Antisuppressors affecting tRNA structure could be either specific or not. For example, if the anticodon recognizing UAA is either IUA or SUA (Yoshida, Takekhi & Ukita, 1971) and the modification (A $\longrightarrow$ I or U $\longrightarrow$ S) is necessary for its recognition, then an antisuppressor affecting modification would be specific for the ochre suppressors (*SUP2*, *SUP11* and *SUPQ5*) but would not affect *SUP11-am*. On the other hand, if the iso-accepting tRNA's are structurally very similar, then they are likely to share certain modifying enzymes, and antisuppressors affecting these would be specific for them (*SUP2*, *SUP11* and *SUP11-am*) but would not affect *SUPQ5*. By the same token, it can be predicted that antisuppressors specific for the two *SUP11* alleles, or quite non-specific, may arise by mutation in genes determining the structure of rare, on the one hand or, on the other, more generally utilized, modifying or maturation enzymes. Antisuppressors specific for *SUP2* or *SUPQ5* would not, of course, appear in our collection.

(3) Several yeast amino-acyl-tRNA synthetases have been isolated and purified: seryl-, valyl- and lysyl-tRNA synthetases all appear to be homogeneous (Makman & Cantoni, 1965; Lagerkvist & Waldenström, 1865) and a temperature-sensitive mutation in the structural gene for isoleucyl-tRNA synthetase results in 99 % loss of the enzyme activity (Hartwell & McLaughlin, 1968). It seems, therefore, that each iso-accepting group of tRNA species is recognized by a single amino-acyl-tRNA synthetase. If an antisuppressor were mutant in the locus determining the structure of tyrosyl-tRNA synthetase, it would be expected to affect only *SUP2*, *SUP11* and *SUP11-am*, but not *SUPQ5*.

(4) Little is known about the control of production of tRNA. If the amount of tRNA were to affect suppression (perhaps by reducing the competition with termination factors), mutations reducing the production of tRNA might be specific or non-specific antisuppressors, depending on whether it is the control of total tRNA, of a set of iso-accepting tRNAs or of a single locus which is affected.

2. MATERIALS AND METHODS

(i) *Yeast strains*

The ochre alleles *ade2–1, can1–100, his5–2 and lys1–1* and the amber allele *trp1–1* were used. All of the antisuppressor strains had the genotype *a* or *α asu, ade2–1, can1–100, lys1–1, his5–2, trp1–1* [*psi*$^-$]. The other strains were:

Strain no.	Genotype	Phenotype
X2182	a *SUP2, ade2–1, can1–100, lys1–1, his 5–2, trp5–48, leu1–12, met1–1,* [*psi*$^-$]	Requiring leucine and methionine for growth
367/8b TRP	*α SUPQ2-am, ade2–1, trp1–1* [*psi*–]	Requires adenine for growth
SM135/1c	a *SUPQ5, ade2–1, can1-100, lys1–1, trp1–1* [*psi*+]	Requires tryptophan for growth
219/7b	*α ade2–1, can1–100, lys1–1, his5–2* [*psi*$^-$]	Requires adenine, lysine and histidine for growth and is resistant to canavine

We are indebted to the Yeast Stock Centre, University of California, Berkeley for strain X2182.

(ii) *Media and genetic analysis*

These have been previously described (McCready & Cox, 1973).

3. RESULTS

(i) *Effect of the antisuppressors on* SUP2

Table 1 gives the results of tetrad analysis from crosses to X2182, giving the following diploid genotype:

$$\frac{asu \quad + \quad ade2.1\ can1.100\ lys1.1\ his5.2\ trp1.1 \quad + \quad +}{+ \quad SUP2\ ade2.1\ can1.100\ lys1.1\ his5.2\ trp1.1\ leu1.12\ met1.1} \quad [\text{psi}^-]$$

Where several antisuppressor alleles at the same locus have been tested, the results have been pooled. Omission plates, on which the phenotypes were tested, were examined after two and after four days' incubation at 28 °C and growth recorded as ' + ' (growth) or ' − ' (no growth detectable). Where growth appeared after four days' incubation, there having been none after two, this has been recorded. Since the diploids were all heterogyous for both the antisuppressor locus being tested and for *SUP2*, it is expected that, if the antisuppressor has no effect on the suppressor, all suppressible phenotypes would segregate *2 suppressed*:*2 non-suppressed* spores in each tetrad. If the antisuppressor has an effect, a proportion of tetrads will segregate no (0:4) or only one (1:3) suppressed spore. The Table shows that all the antisuppressors were able to prevent suppression by *SUP2* of

Table 1. *Results of tetrad analysis of crosses involving* SUP2 *and antisuppressors* (*all* [psi⁻])

Antisuppressor alleles	Marker	Days of growth on test plates	No. of tetrads with suppressed: non-suppressed spores			Spores suppressed: non-suppressed	Spores suppressed (%)
			0:4	1:3	2:2		
asu1, (*asu1.1*, *1.2* *1.3*, *1.4*)	*ade2.1*	4	3	23	9	41:99	29
	can1.100	4	1	23	11	45:95	32
	lys1.1	2	0	0	35	70:70	50
	his5.2	2	1	5	29	63:77	45
	his5.2	4	0	0	35	70:70	50
asu2 (*asu2.2*, *2.3*)	*ade2.1*	4	2	10	2	14:42	25
	can1.100	4	2	10	2	14:42	25
	lys1.1	2	0	0	14	28:28	50
	his5.2	2	1	5	8	21:35	38
	his5.2	4	0	0	14	28:28	50
asu 3 (*asu3.3*, *3.4*, *3.5*)	*ade2.1*	4	4	17	6	29:79	27
	can1.100	4	4	17	6	29:79	27
	lys1.1	2	0	0	27	54:54	50
	his5.2	2	2	9	16	41:67	38
	his5.2	4	0	0	27	54:54	50
asu4 (*asu4.1*, *4.2*)	*ade2.1*	4	6	8	5	18:58	24
	can1.100	4	6	8	5	18:58	24
	lys1.1	2	0	0	19	38:38	50
	his5.2	2	2	3	14	31:45	41
	his5.2	4	0	2	17	34:40	45
asu5 (*asu5.1*, *5.2*)	*ade2.1*	4	1	15	7	29:63	32
	can1.100	4	1	15	7	29:63	32
	lys1.1	2	0	0	23	46:46	50
	his5.2	2	0	0	23	46:46	50
asu6 (*asu6.1*)	*ade2.1*	4	2	4	0	4:20	17
	can1.100	4	2	4	0	4:20	17
	lys1.1	2	0	0	6	12:12	50
	his5.2	2	0	0	6	12:12	50
asu7 (*asu7.1*, *7.2*)	*ade2.1*	4	5	8	4	16:52	24
	can1.100	4	5	8	4	16:52	24
	lys1.1	2	0	0	17	34:34	50
	his5.2	2	0	0	17	34:34	50
asu8 (*asu8.1*)	*ade2.1*	4	0	0	0	9:27	25
	can1.100	4	0	9	0	9:27	25
	lys1.1	2	0	2	7	16:20	45
	lys1.1	4	0	0	9	18:18	50
	his5.2	2	0	5	4	13:23	36
	his5.2	4	0	0	9	18:18	50
Control: (X2182 × 219/7b)	*ade2.1*	4	0	0	9	18:18	50
	can1.100	4	0	0	9	18:18	50
	lys1.1	2	0	0	9	18:18	50
	his5.2	2	0	0	9	18:18	50

ade 2.1 and *can 1.100*, but not of *lys1.1*. Suppression of *his5.2* was not prevented either, but in the presence of certain alleles of *asu1*, *asu2*, *asu3*, *asu4* and *asu8*, it was delayed. Segregants from some crosses included cultures in which *his5.2* (and in one cross, *lys1.1*) was not suppressed after two days' incubation, but was after four days (Table 2).

Table 2. *The delay in the expression of* SUP2 *as a suppressor of* his5.2 *as a result of the presence of antisuppressor alleles*

Anti-suppressor allele	Total spore cultures	No. not suppressed (*SUP+*)	No. suppressed after 4 days' incubation (*SUP2*)	No. of these not suppressed after 2 days' incubation
1.1	36	18	18	9
1.2	44	22	22	0
1.3	20	10	10	0
1.4	40	20	20	0
2.2	24	12	12	0
2.3	32	16	16	7
3.3	44	22	22	4
3.4	20	10	10	3
3.5	44	22	22	6
4.1	28	14	14	5
4.2	44	22	22	0
5.1	48	24	24	0
5.2	44	22	22	0
6.1	24	12	12	0
7.1	28	14	14	0
7.2	40	20	20	0
8.1	36	18	18	5

Note. lys1.1 was suppressed after 2 days' incubation in all those cultures in which *his5.2* was suppressed, except that two *SUP2* cultures in which *asu8.1* was present showed no growth after 2 days', but grew after 4 days' incubation.

With the exception of four cultures, all from the cross involving *asu1.2*, there was complete coincidence of suppression and non-suppression of *ade2.1* and *c.n1.100*; that is, all adenine-independent cultures were also sensitive to canavanine.

The non-suppressible markers *leu1.2* and *met1.1* segregated 2:2 in all tetrads and all cultures required tryptophan for growth.

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(ii) *Effect of the antisuppressors on* SUPQ5

The results of tetrad analysis of crosses to SM135/3c are shown in Table 3. All the diploids had the genotype:

$$\frac{asu \quad + \quad ade2.1 \;\; can1.100 \;\; lys1.1 \;\; his5.2 \;\; trp1.1}{+ \;\; SUPQ5 \;\; ade2.1 \;\; can1.100 \;\; lys1.1 \quad + \quad trp1.1} \quad [psi^+]$$

Since, in these diploids, *his5.2* is heterozygous, it is not always possible to be certain whether growth on histidineless plates is due to the activity of the suppressor or occurs because of the presence of the wild-type *his5* allele. The segregation of histidine requirement has therefore been omitted from the Table. The

Table 3. *Results of tetrad analysis of crosses involving* SUPQ5 *and various antisuppressors* (*all* [psi$^+$])

Antisuppressor	Marker	Days of growth on test plates	No. of tetrads with suppressed: non-suppressed spores 0:4	1:3	2:2	Spores suppressed: non-suppressed	Spores suppressed (%)
asu1 (*asu1.1*, *1.2*, *1.3*, *1*).–	*ade2.1*	4	9	22	5	32:112	22
	can1.100	4	8	21	7	35:109	24
	lys1.1	2	6	20	10	40:104	28
	lys1.1	4	1	12	23	58:86	40
asu2 (*asu2.1*, *2.2*, *2.3*)	*ade2.1*	4	8	21	10	41:115	26
	can1.100	4	7	21	11	43:113	28
	lys1.1	2	5	12	22	56:100	36
	lys1.1	4	1	11	27	65:91	42
asu3 (*asu3.3*, *3.4*, *3.5*)	*ade2.1*	4	7	13	7	27:81	25
	can1.100	4	13	13	1	15:93	14
	lys1.1	2	3£	10	14	38:70	35
	lys1,1	4	0	7	20	47:61	44
asu4 (*asu4.1*, *4.2*, *4.3*)	*ade2.1*	4	5	9	7	23:61	27
	can1.100	4	5	11	5	21:63	25
	lys1.1	2	5	7	9	25:59	29
	lys1.1	4	1	6	14	34:50	40
asu5 (*asu5.1*, *5.2*)	*ade2.1*	4	5	4	6	16:44	27
	can1.100	4	1	4	10	24:36	40
	lys1.1	2	0	0	15	30:30	50
asu7 (*asu7.1*, *7.2*)	*ade2.1*	4	1	3	3	9:19	32
	can.100	4	2	4	1	6:22	21
	lys1.1	2	0	2	5	12:16	43
	lys1.1	4	0	0	7	14:14	50
asu8 (*asu8.1*)	*ade2.1*	4	5	4	1	6:34	15
	can1.100	4	2	5	3	11:29	28
	lys1.1	2	6	2	2	6:34	15
	lys1.1	4	0	0	10	20:20	50
Control: SM135/3c × 219/7b	*ade2.1*	4	0	0	8	16:16	50
	can1.100	4	0	0	8	16:16	50
	lys1.1	2	0	0	8	16:16	50

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suppression of adenine and lysine requirements and of canavanine resistance has been recorded as before. Again, it is clear that all the antisuppressors severely interfere with the ability of the suppressor to suppress *ade 2.1* and *can1.100*. In contrast with their effects on *SUP2*, they do not do so indiscriminately: it is

Table 4. *Results of tetrad analyses of crosses of alleles of the* asu4 *locus with* SUPQ5

Allele	Marker	Days of growth	No. of tetrads segregating non-suppressed:suppressed cultures in the ratios: 4:0	3:1	2:2
4.1	*ade2.1*	4	2	4	3
	can1.100	4	1	8	0
	lys1.1	2	2	4	3
	lys1.1	4	1	3	5
4.2	*ade2.1*	4	2	3	0
	can1.100	4	0	0	5
	lys1.1	2	0	3	2
4.3	*ade2.1*	4	1	2	4
	can1.100	4	4	3	0
	lys1.1	2	3	0	4
	lys1.1	4	0	0	7

Table 5. *Antisuppressor alleles which fail to effect the suppression of* (*A*), lys1.1 *or* (*B*) can1.100 *by* SUPQ5

(In crosses involving these alleles, either lysine requirement or canavanine resistance or both segregated 2:2 in all tetrads.)

A	B
1·2	
1·4	1·4
2·2	
3·3	
	4·2
4·3	
5·1	5·1
5·2	
7·1	
7·2	
8·1	

noticeable that when segregating with *asu3* alleles, for example, *SUPQ5* suppresses *ade 2.1* in more cultures than it does *can1.100*, while the reverse is true of its combinations with *asu5* alleles and *asu8.1*. In no set of tetrads involving antisuppressor alleles from any one locus is there complete coincidence of suppression of these markers. However, pooling the data within each locus has obscured the fact that alleles show considerable variation in their effects. This is illustrated in Table 4 in which the tetrads from crosses involving three alleles of *asu4* are

described. Apart from *asu4.2*, two other *asu* mutations fail altogether to interfere with the suppression by *SUPQ5* of *can1.100* (Table 5). On the other hand, three of the four alleles of *asu1* show complete coincidence of suppression of *ade2.1* and *can1.100* among the segregants. Alleles of *asu5*, *asu7* and *asu8* fail to prevent suppression of *lys1.1*, as do some alleles of the other loci (Table 5). However, the remaining antisuppressors tested clearly interfere with the suppressor's activity in many cultures.

All the segregants required tryptophan for growth.

Table 6. *Tetrad data from crosses involving* SUP11am *and antisuppressors*

Antisuppressors	No. of tetrads with trp⁺:trp⁻ spores 0:4	1:3	2:2	Spores suppressed: non-suppressed	Spores suppressed (%)
asu1.1	0	0	9	18:18	50
asu1.2	0	0	5	10:10	50
asu1.4	0	0	12	24:24	50
asu2.2	0	0	3	6:6	50
asu2.3	0	0	8	16:16	50
asu3.1	0	0	4	8:8	50
asu3.2	0	0	1	2:2	50
asu3.3	0	0	2	4:4	50
asu4.1	0	0	6	12:12	50
asu4.2	0	0	5	10:10	50
asu5.1	0	0	12	24:24	50
asu6.1	0	0	7	14:14	50
asu7.1	0	0	8	16:16	50
asu8.1	0	0	4	8:8	50

(iii) *Effect of the antisuppressors on* SUP11am

Table 6 summarizes the results of crosses of the antisuppressors to 367/8b TRP⁺, which produces diploids of genotype:

$$\frac{asu \quad + \quad ade2.1\ lys1.1\ can1.100\ his5.2\ trp1.1}{+\ SUP11am\ ade2.1 \quad + \quad + \quad + \quad trp1.1} \quad [psi^-]$$

No antisuppressor has any effect on the ability of *SUP11am* to suppress *trp1.1*.

4. DISCUSSION

(1) It is noticeable about all the antisuppressors described here that none of them completely prevents suppression by any of the suppressors with which they have been combined. *SUP2* continues to suppress *lys1.1* and *his5.2* in the presence of any antisuppressor and in [*psi*⁺] strains, *SUPQ5* combined with some of the mutations suppresses *lys1.1* occasionally and does so invariably when combined with the others. We have previously shown that *SUPQ2* (*SUP11*) sup-

presses all these ochre mutations when coupled with antisuppressors in a [*psi*$^+$] background: it is however, no longer a recessive lethal. These observations reinforce our earlier conclusion that the antisuppressors depress the level of suppression, rather than completely inactivate the suppressor. This means that no tRNA is removed from production or prevented from maturation completely by any of these suppressor mutations.

(ii) None of the suppressors is specific to the isoinserting suppressors *SUP11* and *SUP2*; both these suppressors and *SUPQ5* are affected. This eliminates the possibility that any of them represents an altered activating enzyme.

(iii) No effect on suppression by *SUP11am* of *trp1.1* could be detected. This may have been because amber suppression is more efficient than ochre suppression (Sherman *et al.* 1973) and that a reduction in suppressor efficiency would go undetected in the system chosen. However, if the antisuppressors are indeed all ochre-specific, this would suggest that they affect codon recognition and that the loci involved determine the structure or amounts of certain ribosomal proteins, of ochre-specific termination factors or of enzymes affecting tRNA structures or bases that are involved in the recognition of ochre codons. The fact that eight different loci can mutate to have this effect makes the last two possibilities rather unattractive explanations.

If a more sensitive test shows that amber suppression is indeed affected, this would enlarge the range of possible modes of action of the antisuppressors to include factors involved in total tRNA production; tRNA maturation; the modifications of tRNA bases required generally for tRNA function; ribosomal structures that affect tRNA binding and conformation and possibly polypeptide chain elongation and termination factors. At this point no more firm conclusion can be reached.

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