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Antisuppressor mutations in *Aspergillus nidulans*: cold-resistant revertants of suppressor *suaC109*

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Summary

Cold-resistant revertants of the cold-sensitive, ribosomal suppressor *suaC109* have been isolated, with a view to obtaining mutations in new ribosomal protein genes. Many revertants had reduced suppressor activity and were classified as antisuppressor mutants. Both intragenic and extragenic reversions were found. In seven strains the extragenic reversion to cold resistance segregated with the antisuppressor phenotype, and these were designated *asu* mutations. Three of the five *asu* genes, C, B and D were mapped to linkage groups, I, II and V respectively. The antisuppressors are not gene-specific, although they mainly antagonize the activity of ribosomal suppressors. The antisuppressors altered all aspects of the phenotype of suppressor *suaC109* including sensitivity to aminoglycoside antibiotics, and are therefore thought to be mutations in ribosomal protein genes.

1. Introduction

Ribosomes are more complex and more difficult to study in eukaryotes than prokaryotes, because eukaryotes require more components for translation and because their ribosomes cannot be assembled *in vitro*. Fortunately, dissection of the eukaryotic ribosome can be carried out indirectly by means of genetic techniques.

Direct selection methods for ribosomal mutants with an obviously altered phenotype (drug resistance or suppressor activity, for example) can only be expected to yield mutations in a limited number of genes. One indirect selection method, which could be used to extend the number of identifiable genes, is that of reversion of existing ribosomal mutations. This relies on the fact that many ribosomal components must cooperate to maintain the structural and functional integrity of the organelle, and that mutations leading to altered components could be complemented by alterations in otherwise unidentified genes. It may even be possible to use the cooperative and antagonistic effects between mutations altering ribosome structure to identify every structural gene for a ribosomal protein. Suitable pairs of antagonistic phenotypes would be: suppressors and antisuppressors, antisuppressors and allosuppressors, resistance versus hypersensitivity to cold or drugs.

Suppressor and antisuppressor mutants have been isolated and characterized in *Podospora anserina* (Picard, 1973; Picard-Bennoun, 1976; Coppin-Raynal, 1977, 1981, 1982). These mutations are similar to the *ramA* and *ramC* suppressors of *E. coli* and the antagonistic mutations in *strA*, *neaA* and *neaB* (review: De Wilde *et al.* 1977) and *rplF* (Kuhberger *et al.* 1979), which restrict misreading and nonsense suppression. The *su*1 and *su*2 suppressor genes of *Podospora* are particularly interesting, since weak suppressors can cooperate to give stronger suppression and antisuppressors of *su*1 can map in *su*2 and vice versa. Ribosomal suppressors and antisuppressors have been found in other fungi: *Saccharomyces cerevisiae* (review: Sherman, 1982; Masurekar *et al.* 1981; Liebman & Cavenagh, 1980; McReady & Cox, 1973); *Schizosaccharomyces pombe* (Barben, 1966; Hawthorne & Leopold, 1974; Thuriaux *et al.* 1975).

In our initial work with *Aspergillus nidulans*, Roberts, Martinelli & Scazzochio (1979) selected five suppressible alleles in four unrelated loci and seven allele-specific suppressors. Three suppressors had properties associated with ribosomal mutants: cold sensitivity, morphological alterations and hypersensitivity to ribosomal antibiotics (Martinelli, 1984). Two of these were demonstrated to have altered ribosomal profiles in ion-exchange chromatography (Harvey & Martinelli, 1983). Reversion of these ribosomal suppressors could be expected to lead to the isolation of antisuppressors, some of which should in their turn be ribosomal mutations. One of these suppressors, *suaC109*, was chosen for this pilot study. Other suppressors are now being used for the same purpose by Churcher and Martinelli.

suaC109 is on linkage group VII. It suppresses a wide range of alleles including the original five of

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Roberts *et al.* (1979): *alX4*; *sB43*; *alcR125*; *niaD500* and *niaD501*, all of which have the properties of nonsense mutations (Martinelli *et al.* 1984). The *suaC109* mutation is pleiotropic. It slows growth, reduces fertility and conidiation, besides increasing sensitivity to hygromycin, paromomycin, geneticin and cycloheximide (Martinelli, 1984 and unpublished results) and results in poor growth at temperatures below 37 °C. All of these alterations are typical of ribosomal mutants, and so reversal of any facet of this phenotype should lead to the isolation of other ribosomal mutations with compensating phenotypes. Reversion to hygromycin resistance has been attempted (Zamir & Martinelli, 1984) and will be reported fully elsewhere. In this study reversion of the cold-sensitive phenotype has been used, with a view to obtaining a new class of ribosomal mutations. Suppression of *alX4* is very clear on media containing allantoin as nitrogen source, so this medium was used to identify a group of revertants which also contained antisuppressor mutations.

2. Materials and methods

(i) *Genetical techniques*

These were based on those of Pontecorvo *et al.* (1953).

(ii) *Strains*

Cold-resistant revertants were isolated in strain 380 (see Table 1). This contains the suppressible alleles *alX4* in the allantoinase gene and *sB43* in the sulphate permease gene, as well as the allele-specific suppressor *suaC109* (Roberts *et al.* 1979). Full genotypes and strains are given in Table 1. Strain 17 was used as the *alX+* control and 390 as the *alX4*, *sB43* control. Clutterbuck (1974) should be referred to for other gene symbols.

(iii) *Media*

Complete medium (CM) and minimal medium are described in Cove (1966). SC refers to minimal medium containing all the nutritional requirements necessary to compensate background auxotrophic markers, a carbon source which is glucose, a nitrogen source which is sodium nitrate unless specified and thiosulphate instead of sulphate to supplement *sB43* strains. SC allantoin therefore has allantoin as nitrogen source and SC sulphate lacks thiosulphate. These two media are used to score for the presence of *alX4* and *sB43* and their suppression. Supplements are given in Roberts *et al.* (1979). Benlate (Hastie, 1970) or *p*-fluorophenylalanine (McCully & Forbes, 1965) was added to CM to induce haploidization. Filter-sterilized stock solutions of aminoglycoside antibiotics were added to molten CM to give final concentrations of 25 or 50 μM hygromycin or 2 mM paromomycin. The antibiotics were kindly supplied by Eli Lilly and Parke-Davis, respectively.

Table 1. *Strains used in this work*

Current Birkbeck stock number	Mutant stock	Genotype	Origin
17		*pabaA1*	
79		*yA1*; *facA303*; *riboD5*; *pyroA4*	
387		*yA1*; *wA3*; *pantoB100*; *alX4*	
390		*pabaA1*; *fwA1*; *alX4*; *sB43*	
380		*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*	Mutation of 390
381		*biA1*; *ribo-6*; *alX4*; *sB43*; *suaC109*	380 cross
			Mutation of 380[a]
394	CR5a	*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*; *asuA5*	Induced, 30 °C
395	CR11b	*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*; *asuB11*	Induced, 25 °C
401	CR13c	*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*; *asuD13*	Induced, 25 °C
399	CR14a	*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*; *asuD14*	Spontaneous, 30 °C
400	CR15c	*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*; *asuC15*	Induced, 25 °C
398	CR16c	*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*; *asuC16*	Induced, 25 °C
397	CR26b	*pabaA1*; *fwA1*; *alX4*; *sB43*; *suaC109*; *asuE26*	Induced, 25 °C
404		*asuA5*; *alX4*; *sB43*; *yA1*; *pantoB100*	394 × 387
405		*asuC16*; *alX4*; *yA1*	398 × 387
406		*asuD13*; *alX4*; *sB43*; *fwA1*; *pantoB100*; *pabaA1*	401 × 387
407		*asuB11*; *alX4*; *sB43*; *yA1*; *pantoB100*	395 × 387
410		*asuE26*; *alX4*; *sB43*; *fwA1*	397 × 387
423		*asuD14*; *alX4*; *sB43*; *wA3*; *panto B100*	399 × 387
424		*asuC15*; *alX4*; *yA1*; *pantoB100*.	400 × 387

[a] Mutations were induced by exposure to uv light. 30°, 25 °C refer to the temperature at which the mutated conidia were incubated in order to select cold-resistant revertants of 380.

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(iv) *Mutagenesis*

Ultraviolet light was used to induce mutations at a dosage which gave a conidial survival rate of 1%. Treated or untreated conidia of strain 380 were plated on SC ammonium at a range of dilutions. Cultures were incubated at 30, 25 and 20 °C until definite colonies were visible.

(v) *Revertant isolation*

One revertant of each type of morphology was picked per plate. Revertant strains are designated CR for cold resistance, then 1–35 to indicate the plate from which they were isolated and a–f to indicate the individual isolate.

(vi) *Growth measurements*

Conidial suspensions were stabbed into the centre of 5 cm agar plates, in duplicate or triplicate. Colony diameters were measured once or twice a day with a ruler at 2 × magnification. Lag phases and radial growth rates (K_r) were calculated.

(vii) *Scoring of crosses*

Both cold sensitivity and antisuppression phenotypes were easier to score on 17-point replica plates than the usual 25-point ones.

3. Results

(i) *Isolation and initial characterization of cold-resistant revertants of* suaC109

Strain 380 was used for the isolation of cold-resistant revertants of the cold-sensitive suppressor *suaC109*. Reversion of one aspect of the pleiotropic mutation could be expected to affect other phenotypic characters, so that the suppression of *alX4* was also measured. The mutation *alX4* causes very poor growth on SC allantoin. This is improved by the presence of suppressor *suaC109* and could be expected to deteriorate again if an antisuppressor mutation was induced. Thus revertants were replicated to SC allantoin to test for antisuppressor activity.

When plates were seeded with uv-treated or untreated conidia, there was considerable background growth at 30 °C, where hyphal extension and branching of *suaC109* strains are reduced, no growth at 20 °C, where germination takes 3–4 weeks, and intermediate growth at 25 °C. Overall, the spontaneous reversion rate per viable conidium was 3×10^{-5} and the rate induced by ultraviolet light was 3×10^{-3}. Fewer revertants were found at 20 than 30 °C.

A wide range of cold-resistant phenotypes was seen amongst the 46 mutants examined. Half of the revertants grew poorly on SC allantoin (weak antisuppressors) or very poorly (strong antisuppressors), whereas other strains were like strain 380. No completely unsuppressed phenotype was seen. Several of the revertants did not grow well at 37 °C – the normal temperature for strain maintenance – and were excluded from further study.

Little correlation was found between the method of isolation and degree of cold resistance or antisuppressor activity. However, 14 out of 15 spontaneously occurring cold-resistant mutants had antisuppressor activity, whereas the majority of induced mutants did not. Only those revertants isolated at 20 were very cold-resistant at 20 °C.

The term 'cold-resistant' is used here to define the response of a revertant strain to growth at low temperatures, this response being less than that of wild type, but better than that of strain 380, with *suaC109*.

(ii) *Genetical classification of revertants*

To distinguish between intragenic and extragenic reversion of *suaC109*, the CR revertants were crossed to strain 387 (*alX4*, *sua+*). Since the crosses were homozygous for *alX4*, it was possible to score directly the inheritance of *suaC109* or any mutations which modified its expression by replicating progeny to SC allantoin, or alternatively by incubating at low temperature.

Amongst the revertants, certain classes of mutation could be expected. The simplest ones are summarized below, using *cor* to represent a gene mutating only to cold resistance and *asu* for an antisuppressor gene.

(I) *Mutation within* suaC109 *which altered only the cold-sensitive aspect of the phenotype*

Ratio expected: 1 partially cold-resistant suppressor type : 1 cold-resistant unsuppressed type (= wild type), i.e. 1 *suaC′* : 1 *suaC+*. Examples: none.

(II) *Intragenic* suaC *mutation which affects both the suppressor expression and cold sensitivity*

Ratio expected: 1 partially cold-resistant antisuppressor type : 1 cold-resistant unsuppressed type, i.e. 1 *suaC″* : 1 *suaC+*. Examples: CR13a, 12d, 6a, 32a.

(III) *Mutation at another locus which affected only cold sensitivity*

Ratio expected: 1 cold-sensitive suppressor type : 1 partially resistant suppressor type : 2 cold-resistant unsuppressed types, i.e. 1 *suaC109*, *cor+* : 1 *suaC109*, *cor−* : 1 *suaC+*, *cor+* : 1 *suaC+*, *cor−*. Examples: CR7b, 14b, 16a, 33a.

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(IV) *Extragenic mutation which counteracted both aspects of the* suaC109 *phenotype, cold sensitivity and suppressor activity*

Ratio expected: 1 cold-sensitive suppressor type:1 partially cold-resistant antisuppressor type:2 cold-resistant unsuppressed ones, i.e. 1 *suaC109, asu+* :1 *suaC109, asu−* :1 *suaC+, asu+* :1 *suaC+, asu−*. Examples: CR5a, 11b, 13c, 15c, 16c, 14a, 26b.

In the class IV crosses, all three predicted phenotypes were observed on SC allantoin, i.e. suppressed, antisuppressed and unsuppressed, as well as the three predicted types on plates incubated at low temperatures, i.e. wild-type full cold resistance, complete cold sensitivity and partial cold resistance. Slightly over 50% of the progeny had unsuppressed phenotype on SC allantoin and were presumed to represent two genotypes: *sua+asu+* and *sua+asu−*, with the antisuppressor mutation not identifiable in the absence of the suppressor. This hypothesis was tested by backcrossing six mutant progeny from each cross with the suppressor parent. At least one from each original cross was shown to contain a silent antisuppressor mutation. Thus the partial cold-resistance phenotype was masked by the full wild-type cold resistance expressed by non-suppressor-containing colonies, as was the antisuppressor phenotype. (However, some antisuppressors do affect antibiotic resistance in the absence of *suaC109*, see Section xi.) The ratios obtained in these backcrosses are given in Table 2.

Only the class IV mutants, with extragenic suppressors were studied further. They have been given stock numbers (Table 1), and their phenotypes are illustrated in Fig. 2.

(iii) *Location of antisuppressor mutations to linkage groups*

The seven CR strains containing extragenic *asu* mutations were combined in diploids with master strain MSF (McCully & Forbes, 1966). This strain had been modified by the addition of *alX4*, so that all diploids were homozygous for *alX4*, thus facilitating the scoring of suppressor and antisuppressor mutations. The presence of *suaC109* on linkage group VII allowed scoring of the antisuppressor mutations which have no phenotype in an *sua+* strain. All asu mutations segregated from *suaC109* and were therefore not on linkage group VII. *asu-15* and *asu-16* were located to linkage group I, *asu-13* and *asu-14* to linkage group V and *asu-11* to II. The mutations, *asu-26* and *asu-5*, were not unequivocally located.

(iv) *Allelism tests between* asu *mutations*

The seven antisuppressor strains were crossed in all possible pairwise combinations so that the number of antisuppressor genes could be determined. The crosses were made between the following types of strain:

alX4; *asuY−*; *suaC109* × *alX4*; *asuZ−*; *suaC+*

where Z and Y represent different antisuppressor mutations. In particular, progeny were scored both for levels of suppression and for cold sensitivity. A cross between allelic antisuppressors should give rise to three types of progeny, those phenotypically *asuY−* and those *asuZ−*, i.e. parentals and an unsuppressed class (*alX4*; *sua+*; *asu+/asu−*). Non-allelic mutations should give rise to an additional class. On SC allantoin this would be suppressed (*alX4*;

Table 2. *Crosses between strains with extragenic antisuppressor mutations* (asu−, suaC+) *and suppressor strain 381* (asu+, suaC109)

Segregation of phenotypes and genotypes

Cr strain	asu allele	Anti-suppressed *asu−* *sua−*	Suppressed *asu+* *sua−*	Unsuppressed[a] *asu+* or *asu−* *sua+* *sua+*	Total progeny scored
5a	*asuA5*	23 (46)[b]	17 (17)[b]	38	78 (63)[b]
11b	*asuB11*	81	80	183	344
13c	*asuD13*	37	30	102	169
14a	*asuD14*	29	28	42	99
15c	*asuC15*	34	31	77	142
16c	*asuC16*	30 (46)[b]	31 (72)[b]	37	98 (118)[b]
26b	*asuE26*	32	30	60	122

Antisuppressor strains were obtained as progeny in previous outcrosses. General genotype: −asu−; pantoB100; alX4; sB43; fwA/yA/wA, see Table1.

[a] These classes are indistinguishable without doing back-crosses.

[b] Only progeny which could utilise allantoin were selected. Normally a random sample of progeny was analysed.

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suaC109; *asu*+) in phenotype. The presence of the latter is critical proof of non-allelism. Only sufficient progeny to detect the presence of this important class was scored (50–100), except where allelism was suspected when more progeny were scored.

asu-13 and *asu-14* were judged to be allelic after analysing 153 progeny, similarly for *asu-15* and *asu-16* with 332 progeny. In all other cases, suppressed phenotypes appeared in reasonable numbers, indicating that the suppressors were not allelic. All the gene assignments are given in Tables 1 and 2 and will be used hereafter.

The phenotype of double antisuppressor strains is unknown, since no new phenotype was recognized. Many progeny have been outcrossed to try and identify double mutant strains, but without success. It is possible that they were not found because a doubly mutant strain is inviable.

(v) *Linkage analyses with antisuppressor mutations*

CR14a, containing *asuD14*, was crossed to strain 79 containing *riboD5*, a marker on linkage group V. Linkage of 17 cM between the genes was found with 228 progeny analysed, *asuC15* and *asuC16* are unlinked to the right-arm proximal markers on group I, i.e. *pabaA*, *hisB*, *yA*.

(vi) *Dominance tests with antisuppressor mutations*

The level of dominance of the *asu* mutations was scored in diploids homozygous for *suaC109*. The antisuppressor and cold-sensitive phenotype varied according to whether singly or multiply inoculated plates were used. Cold resistance was mainly semi-dominant. Antisuppressor phenotype was dominant in some cases, recessive in others. There was no correlation with other properties of these mutations.

(vi) *Are the antisuppressors specific for* suaC109?

In order to find out whether the antisuppressor mutations were allele-specific or gene-specific reversions, or alternatively antisuppressor mutations of a more general kind, they were crossed to six of the seven previously described translational suppressors (Martinelli, 1984) that were isolated by Roberts *et al.* (1979). The putative tRNA mutations *suaB111*, *suaD103* and *suaD108* do not cause cold sensitivity, so that only the antisuppression effects of *asu* mutations can be scored. *suaC115* has almost the same properties as *suaC109*. *suaA101* is a very similar ribosomal suppressor, but less sensitive to cold and drugs. Crosses were performed between the following types of strain:

alX4; *sB43*; *sua*−; *asu*+ × *alX4*; *sB*+/−; *sua*+; *asu*−.

None of the antisuppressor mutations is linked to any of the suppressor mutations, so that only 50–100 progeny were anlaysed in order to find at least one *sua*−, *asu*− progeny colony.

As a group, antisuppressors were most active against suppressors *suaA101* and *suaC115* (Table 3). Three of the genes appear to affect ribosomal suppressors only, whereas the other two act on all types. It is not surprising that only half of the *asu* mutations reverse the cold sensitivity of *suaA101*, since this suppressor is only clearly cold-sensitive at 20 °C and the *asu* mutations only confer weak cold resistance on *suaC109* at this temperature. The different results obtained with the very similar alleles at the *asuC* locus

Table 3. *Antisuppressors and their action on other suppressors*

Antisuppressor mutation	*suaA101*			*suaC115*			*suaD108*[a] *suaD103*[b]		*suaB111*	
	1	2	3	1	2	3	1	2	1	2
asuA5	+	+	−	+	+	+	−	−[a, b]	−	−
asuB11	+	+	?	+	−	+	−	−[b]	+	+
asuC15	−	−	−	−	−	+	−	−[a, b]	−	−
asuC16	+	+	+	+	+	+	−	−[a, b]	−	−
asuD13	+	−	−	Infertile			+	+[b]	−	−
asuD14	+	+	+	+	?	+	−	−[b]	−	+
asuE26	−	−	−	+	?	+	−	−[a, b]	Infertile	

Recombinants were scored on SC allantoin (1) and SC sulphate (2) to measure their antagonistic activity on suppressors and on CM at 30 °C and 25 °C (3) to measure their reversal of any cold-sensitive phenotype.

? Effect of antisuppressor is marginal or non-existent, but difficult to judge.

− No antisuppression, *or* no alleviation of cold sensitivity.

+ Antisuppression *or* cold resistance of antisuppressor overrides that of the suppressor.

N.B. *suaB* and *suaD* alleles are not cold sensitive, so this phenotype associated with antisuppressors cannot be scored.

[a] Cross to *suaD108*; [b] Cross to *suaD103*.

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gives a clear indication that these are hetero-alleles; similarly for the *asuD* alleles. From each cross, putative *asu*− *sua*− progeny were selected and crossed to strain 380 to check for the presence of an antisuppressor mutation. This was particularly important in the case of the negative results, when the antisuppressor had no apparent effect on the suppressor.

(vii) *Colony morphology of antisuppressor strains*

suaC109 strains have smaller colonies than wild type on all media and an abnormal morphology. On CM and SC medium at 37 °C, the antisuppressor strains have normal morphology. At 30 °C they have either reduced branching or reduced radii, and at lower temperatures they all have smaller sparser colonies than normal. Suppressor colonies are greatly reduced in density and diameter at lower temperatures (Table 4 and Fig. 2*b*).

On SC allantoin, *alX4* strains have very thin, small colonies due to the non-utilization of allantoin on the one hand and its toxicity on the other. The addition of *suaC109* improves the density, conidiation and radial growth. The further addition of *asuC* mutations does not alter the morphology but reduces the radius slightly. The other antisuppressors considerably reduce the radius and alter the morphology to a fluffy small colony which is very tall and shaped like a top hat.

The other suppressible allele, *sB43*, can also be used to assay the antisuppressor activity. On SC sulphate, the *sB43* mutation causes a very sparse colony, smaller than wild type. The suppressor restores the density to normal, then the addition of antisuppressors restores the radius to wild-type size. The significant change caused by the antisuppressors is the decrease in colony density again.

(ix) *Radial growth rates*

When the growth rates (K_r) were measured on SC and SC allantoin at 37 °C, the results were in broad agreement with the colony morphologies except that the *asuC* mutations caused a slower hyphal extension rate than the other group of antisuppressors on SC allantoin. All antisuppressor strains grew more slowly than the suppressor strain, which was itself slower than wild type, but they were all faster than the *alX4* mutant (Fig. 1*c*).

On SC or CM at 30 °C, some antisuppressors gave growth rates intermediate between suppressor and *sua*+ strains, but *asuC* and *asuD13* strains were quite unaffected by the drop in temperature. At 25 °C, the lag phases were increased in every case and the growth rates were lower. *asuA5* and *asuE26* did not improve the cold sensitivity due to *suaC109* at 25 °C (Fig. 1*b*). On SC allantoin at 30 °C, the antisuppressor strains grew faster than they did at 37 °C and had similar K_r to the *alX*+ strain. It may be possible to explain this peculiar result by *in vitro* studies on suppressor and antisuppressor ribosomes, or by following ribosome biogenesis. No satisfactory explanation can be offered here.

On SC sulphate, the antisuppressor colonies were faster than both the mutant and suppressor colonies (Fig. 1*d*). The *asuC* strains were most similar to the suppressor strain.

Table 4. *Reversal of the phenotype of* suaC109 *by antisuppressor mutations*

		Growth on various media at 37 °C				
CR strain	*asu* mutation	SC allantoin (1)	SC sulphate (2)	CM PAROMO* (1+2)	CM HYGRO* (1)	CM at 25 °C (1+2)
5a	*asuA5*	++	++	++	++	+
11b	*asuB11*	++	++	+	++	+
13c	*asuD13*	+	++	++	++	++
14a	*asuD14*	+	++	++	++	+
15c	*asuC15*	+++	++	+	+	++
16c	*asuC16*	+++	+++	+	+	++
26b	*asuE26*	+	++	+	+	+
380	none	+++	+++	+	+	−
390	none	−	+	+++	+++	+++

Qualitative assessment of strains on replica plates containing 17 colonies. Antisuppressor strains with the genotype *alX4*; *sB43*; *suaC109*; *asu*- were compared with the suppressor strain 380 and the non-suppressor control 390.

* 2 mM paromomycin, 50 μM hygromycin.

(1) Judged by radius and morphology.

(2) Judged by hyphal and conidial density.

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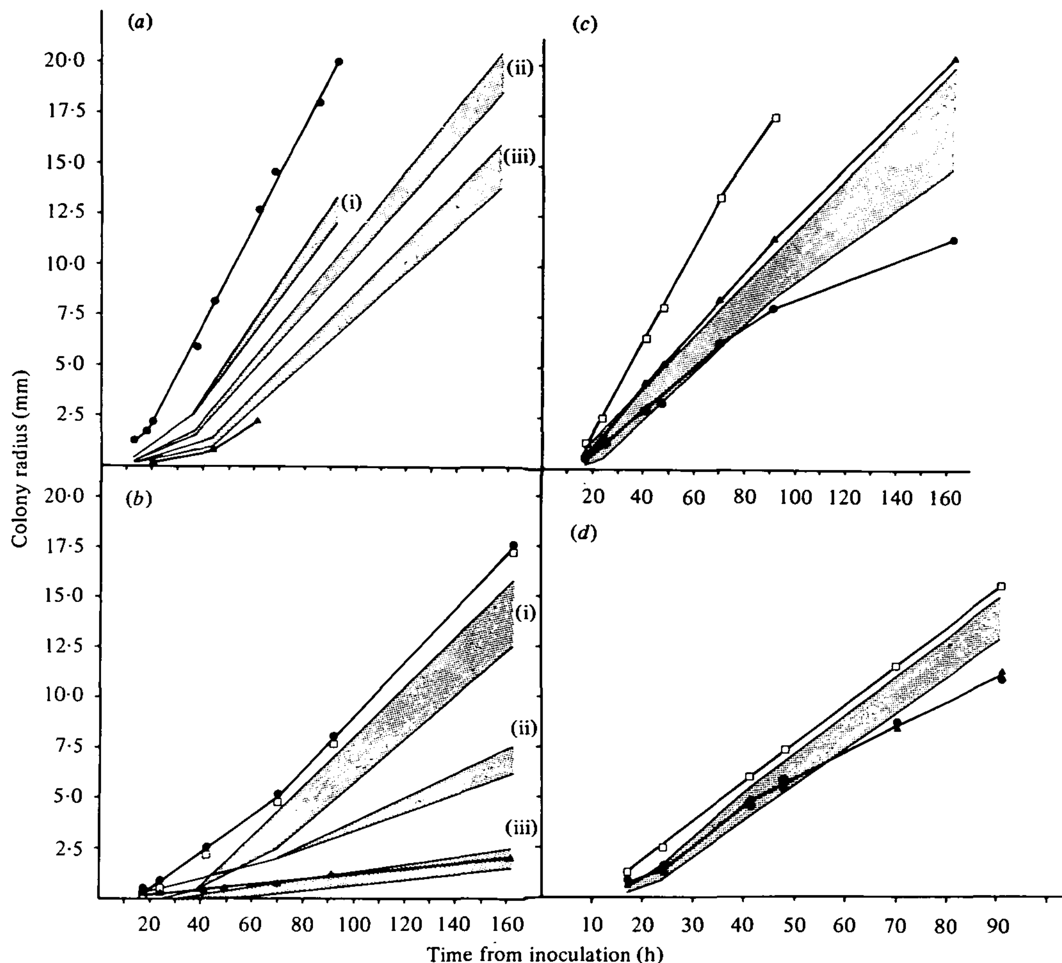

Fig. 1. Effect of antisuppressor mutations on the growth of suppressor strain 380 on antibiotics and at low temperatures, and their antisuppressive activity assayed against *alX4* and *sB43*. (*a*) CM plus 25 μM hygromycin, 37 °C. Antisuppressor strains contained the following mutations, in descending order relative to the graph: (i) *asuD13* and *asuD14*; (ii) *asuE26*, *asuA5* and *asuB11*; (iii) *asuC15* and *asuC16*. (*b*) SC medium at 25 °C: (i) *asuC16*, *asuC15* and *asuD13*; (ii) *asuD14*, *asuB11*; (iii) *asuA5* and *asuE26*. (*c*) SC allantoin at 37°. (*d*) SC sulphate at 37°. ●—●, Strain 390 (*alX4*, *sB43*, *asu+*, *sua+*); □—□, strain 17 (*alX+*, *sB+*, *asu+*, *sua+*); ▲—▲, strain 380 (*alX4*, *sB43*, *asu+*, *suaC109*); ▩, antisuppressor strains (*alX4*, *sB43*, *asu−*, *suaC109*).

(x) *Sensitivity to aminoglycoside antibiotics*

suaC109 strains are hypersensitive to hygromycin and paromomycin, compared with strains 390 and 17 (Martinelli, 1984). Therefore, the antisuppressor strains were tested to see if they relieved this hypersensitivity. With the exception of antisuppressor strains containing *asuC* mutations, all the antisuppressors were reasonably resistant to aminoglycosides compared with the suppressor strain, but did not attain a wild-type K_r (Fig. 1*a*). On the small plates used in these tests, the *asuC* strains were more resistant than the suppressor strain 380, but on multiply inoculated normal plates they were indistinguishable from it (Fig. 2).

In diploids homozygous for *suaC109* and heterozygous for one of the antisuppressor mutations, the paromomycin phenotype was recessive, whereas the hygromycin phenotype attributable to the *asu* mutation was semi-dominant.

(xi) *Growth and morphology of* asu− sua+ *strains*

On SC, CM, SC allantoin and SC sulphate at any temperature, these strains have the same colony appearance as the mutant strain 390 (where appropriate), so that they are indistinguishable from *asu+*, *sua+* strains. Following the discovery that *asuC*, *suaC109* strains were hypersensitive on aminoglycosides, all the *asu−*, *sua+* strains were plated on both drugs. *asuC* strains alone were still hypersensitive, whereas the other antisuppressor strains had wild-type growth rates. *asuA5* and *asuE26* increased the lag phase.

(xii) *Correlation of the properties of antisuppressor strains*

The antisuppressors fall into two broad classes, based on the phenotypes on SC allantoin, SC and CM at various temperatures and aminoglycoside additives

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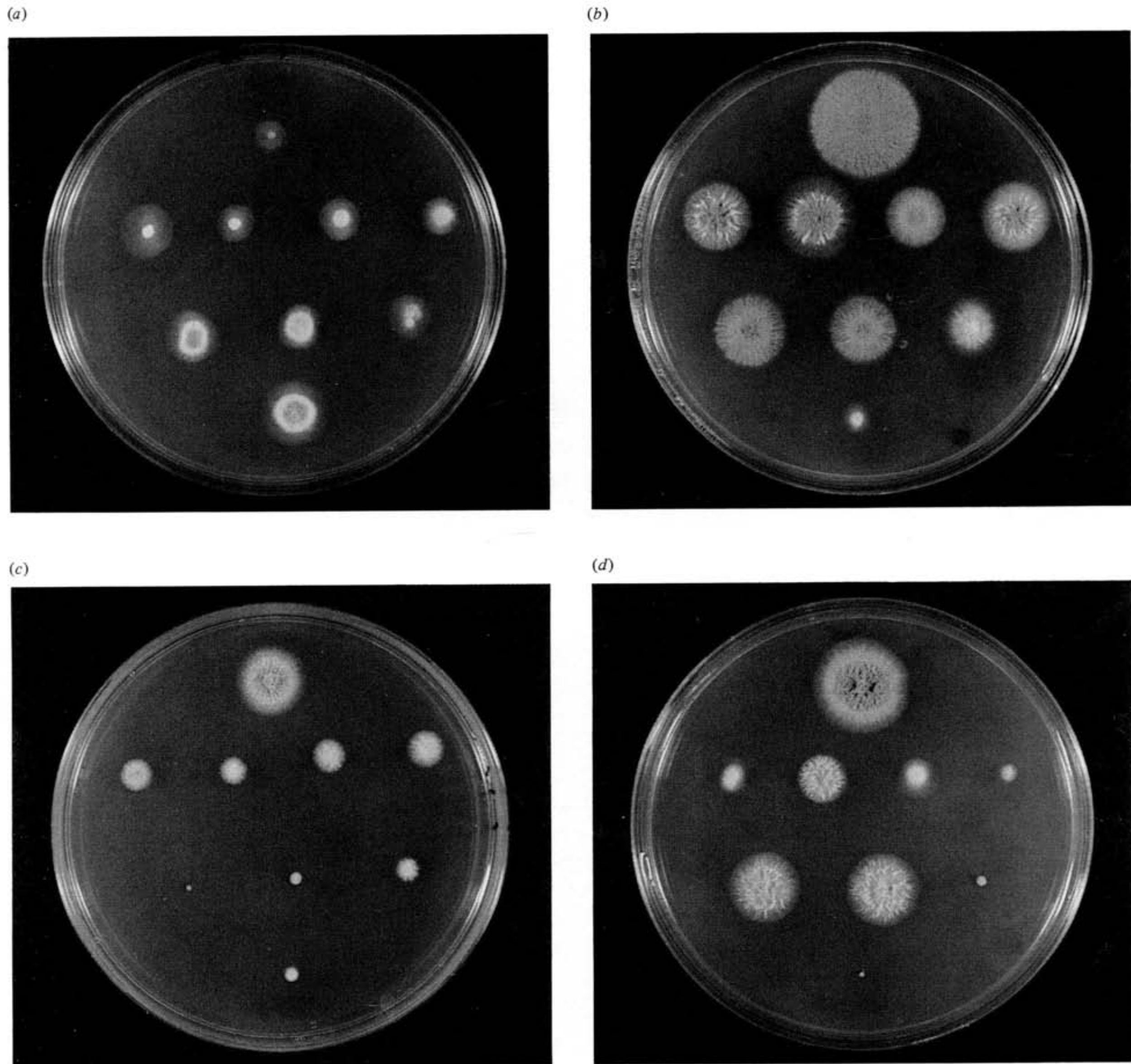

Fig. 2. CR strains containing extragenic antisuppressor mutations. (*a*) SC allantoin at 37 °C; (*b*) CM at 30°; (*c*) CM plus 50 μM hygromycin at 37 °C; (*d*) CM at 25°. Top: 390 (*alX4*; *sB43*; *sua+*; *asu+*); bottom: 380 (*alX4*; *sB43*; *suaC109*; *asu+*); the rest are CR strains with the genotype of 380 plus *asu* mutations. Row 1, left to right: *asuD14*, *asuD13*, *asuB11*, *asuA5*. Row 2, left to right: *asuC15*, *asuC16*, *asuE26*.

(Table 4). These are: the *asuC* mutations and the rest. The *asuC* mutations are the least effective in correcting the phenotype caused by *suaC109*.

4. Discussion

The rationale of isolating cold-resistant revertants as a means of isolating antisuppressors has been confirmed. Both intragenic and extragenic reversions were found and in many cases the mutation was inseparable from a decrease in suppressor activity against *alX4* or *SB43*. Although the intragenic ones were much more cold-resistant than the extragenic ones, they have not been studied further.

It was argued that the specificity of action of the antisuppressors might give a clue to their nature. For instance, one type of antisuppressor mutation alters an enzyme which normally modifies a suppressor tRNA (Laten, Gorman & Bock, 1980). This would not be expected to react with ribosomal suppressors, whereas ribosomal antisuppressors are known to affect both ribosomal (Rosset & Gorini, 1969) and tRNA suppressors in *E. coli* (Strigini & Gorini, 1970). The restrictive or antisuppressor mutations in *strA* (coding for protein S12, Ozaki, Mizushima & Nomura, 1969) antagonize suppression of nonsense mutations by tRNA suppressors or *ram* mutations (for examples see Cabezon *et al.* 1976). The same is true for restrictive mutations in *neaA* (= S17, Bollen *et al.* 1976) and *rpfL* (= L6, Kuhberger *et al.* 1979). All the antisuppressors, except *asuC15*, were allele-unspecific, in that they acted on another allele at the

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suaC locus and on suppressor mutations at other loci. Whereas they most commonly acted on ribosomal suppressors, in rare cases they also antagonized the activity of putative tRNA suppressors (*suaB* and *suaD* alleles). This alone indicates that they might be in ribosomal genes rather than tRNA genes or those coding for modifying enzymes.

The nature of the genes which can be mutated to antisuppressors is at present unknown, but can be conjectured. Two of the antisuppressors, *asuC15* and *asuC16*, have a recognizable phenotype in the absence of *suaC109*. They confer sensitivity to hygromycin and paromomycin. These two antibiotics primarily act on ribosomes and interfere with the elongation of protein chains. Hygromycin in particular is known to block translocation (Gonzalez *et al.* 1978) and both cause misreading *in vitro*. The *asuC* mutations are therefore likely to map in a gene which codes for ribosomal components. *suaC109* certainly alters ribosomes, since 40 S subunit proteins have a different profile from wild type (Harvey & Martinelli, 1983), and *suaC109* ribosomes have a higher misreading level *in vitro* than the control strain (Zamir & Martinelli, unpublished results). Yet other *suaC* alleles have been demonstrated to alter electrophoretic mobility of a ribosomal protein (Bratt & Martinelli, unpublished results). All the antisuppressor mutations can reverse to some extent almost every aspect of the deranged *suaC109* phenotype, for instance hypersensitivity to antibiotics, cold sensitivity, altered morphology and viability. This suggests that all the antisuppressor mutations are in genes which code for ribosomal components. The fact that they can act on both ribosomal and tRNA suppressors does not detract from this argument (see above).

Most of the antisuppressor mutations isolated in *Podospora anserina* by M. Picard-Bennoun and her colleagues (for example: Coppin-Raynal, 1982) have altered ribosomes. Several have been allocated to particular protein genes by two-dimensional electrophoresis (Dequard-Chablat, 1985*a*, 1986). Although more and more cases are appearing in which structural changes in rRNA are responsible for ribosomal phenotypes such as thiostrepton resistance in *Streptomyces azureus* (Cundliffe & Thompson, 1979), or a suppressor mutation mapping in the 15 S rRNA gene of yeast mitochondria (Kruszewska & Slonimski, 1984), it is unlikely that the antisuppressor genes could code for rRNA in *Aspergillus*. The examples of rRNA mutations given here can only be detected because of the single-copy nature of the rRNA gene.

I hope to test these hypotheses by performing two-dimensional electrophoresis to see whether the antisuppressor mutations cause a ribosomal protein to migrate differently. It is also possible that they may decrease the misreading of mRNA *in vitro*. Whatever the outcome, I shall use these antisuppressors to isolate new suppressor loci as has been done in *Podospora* (Dequard-Chablat, 1985*b*), or yeast (Kohli *et al.* 1980). It should be possible to isolate further antisuppressor genes by mutation of *suaC109* and other suppressors and selecting for compensation of other aspects of the pleiotropic phenotype such as the hypersensitivity to antibiotics.

The seven extragenic cold-resistant, antisuppressor mutations mapped in five genes which were scattered in the genome. None of them mapped in suppressor genes. If all these *asu* genes do in fact code for ribosomal proteins, screening for antisuppressors could be a more productive way of uncovering ribosomal genes than screening for suppressors, since this has only revealed two genes so far.

This work was reported briefly by Martinelli (1984).

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