AN ANTISUPPRESSOR THAT ACTS ON OMNIPOTENT SUPPRESSORS IN YEAST

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ABSTRACT

Six partially dominant antisuppressors were obtained that reduce the efficiency of two omnipotent yeast suppressors, *sup45* and *sup35*, thought to be ribosomal ambiguity mutations. Each of these six antisuppressors was shown to fall within a single Mendelian locus, named *asu9*. The *asu9* mutations are specific for omnipotent suppressors; they have no effect on several dominant tRNA-like suppressors. In the absence of suppressors, *asu9* causes sensitivity to the aminoglycoside antibiotic, paromomycin. The properties of *asu9* are consistent with the hypothesis that *asu9* alters yeast ribosomal proteins.

MANY nonsense suppressors have been isolated in *Saccharomyces cerevisiae*, and they have been divided into four major groups on the basis of their codon specificity (reviewed by HAWTHORNE and LEUPOLD 1974). Three of these suppressor types, ochre-specific, amber and umber suppressors, are probably mutant tRNA species (PIPER *et al.* 1976)) that are able to recognize one of the nonsense codons UAA, UAG or UGA, respectively, and to translate it as sense. While the suppressors in these three groups are all dominant or semidominant, suppressors in the fourth group are generally recessive. These recessive suppressors are unusual in their ability to recognize all three nonsense codons and have thus been named omnipotent.

The recessive suppressors have been shown to fall into two complementation groups (INGE-VECHTOMOV and ANDRIANOVA 1970; HAWTHORNE and LEUPOLD 1974; GERLACH 1975), and the two loci identified by HAWTHORNE have been mapped and named *sup35* and *sup45*. Since these suppressors are usually recessive and act on all types of nonsense codons, it is generally believed that they are not mutant tRNAs. Rather, they are thought to involve mutations in some other component of the protein synthesizing apparatus, possibly ribosomal proteins. Indeed, biochemical evidence suggests that the omnipotent yeast suppressors code for a protein affecting the termination of peptide chains on ribosomes. A recessive yeast suppressor has been shown to cause the accumulation of 80S ribosomes that carry unreleased peptidyl tRNA (SMIRNOV *et al.* 1976). Furthermore, SMIRNOV *et al.* (1978) have recently shown that yeast strains containing one of the recessive suppressor mutations have an increased amount of the L30 protein in their 60S ribosomal subunits. Thus, it appears that the omnipotent suppressors

in yeast are analogous to the *ram* mutation in *E. coli*, in which an altered 30S ribosomal protein causes misreading of all three nonsense codons (ROSSET and GORINI 1969; ZIMMERMAN, GARVIN and GORINI 1971; GORINI 1970).

Additional genes involved in protein synthesis can be obtained by selecting for mutations that modify the efficiency of nonsense suppressors. A variety of such modifiers that enhance or reduce suppression efficiency have been isolated in both prokaryotes and eukaryotes (STRIGINI and GORINI 1970; GORINI 1969; GARVIN and GORINI 1975; OZAKI, MIZUSHIMA and NOMURA 1969; THURIAUX *et al.* 1975; PICARD-BENNOUN 1976; COPPIN-RAYNAL 1977). In *Saccharomyces cerevisiae*, a non-Mendelian psi+ factor (COX 1965, 1971; YOUNG 1975; LIEBMAN and SHERMAN 1979), as well as Mendelian allosuppressors (COX 1977), enhance the efficiency of ochre suppressors. In contrast, antisuppressor mutations reduce the efficiency of ochre (MCCREADY and COX 1973; INGE-VECHTOMOV 1967; LATEN, GORMAN and BOCK 1978) and amber (HAWTHORNE 1967; HAWTHORNE and LEUPOLD 1974) suppressors.

In this paper, we describe the isolation and genetic characterization of an antisuppressor, *asu9*, capable of reducing the efficiency of omnipotent (presumed ribosomal) yeast suppressors. All previously isolated antisuppressors in *Saccharomyces cerevisiae* were selected for their ability to reduce the efficiency of the altered tRNA type of suppressor.

MATERIALS AND METHODS

Genetic markers

Suppressible markers: The properties of the suppressible nutritional or resistance markers used in this study are described in LIEBMAN, SHERMAN and STEWART (1976) and are listed in Table 1.

Dominant suppressors: *SUP7-a* and *SUP52-a* suppress only amber (UAG) markers and cause the insertion of tyrosine (SHERMAN *et al.* 1973) and leucine (LIEBMAN *et al.* 1977), re-

TABLE 1

Properties of nonsense mutants

Mutant	Phenotype	Suppressibility	Reference
met8–1	Requires methionine	UAG	HAWTHORNE (1969a)
*tyr7–1**	Requires tyrosine and phenylalanine	UAG	HAWTHORNE (1969a,b)
trp1–1	Requires tryptophan	UAG	HAWTHORNE (1969a,b)
ade3–26	Requires adenine and histidine	UAG	JONES (1972)
ilv1–1	Requires isoleucine	UAG	ROMAN and JACOB (1958)
can1–132	Resistant to canavanine	UAG	SHERMAN (unpublished)
cyc1–76	Lacks iso-1-cytochrome *c*	UAG	STEWART and SHERMAN (1973)
cyc1–72	Lacks iso-1-cytochrome *c*	UAA	SHERMAN and STEWART (1974)
leu2–1	Requires leucine	UAA	HAWTHORNE (1969a,b)
lys1–1	Requires lysine	UAA	GILMORE and MORTIMER (1966)
lys2–1	Requires lysine	UAA	HAWTHORNE (1969a,b)
his5–2	Requires histidine	UAA	GILMORE and MORTIMER (1966)

* or *aro7–1*.

spectively, at the site of the amber codon. *SUP7-o* suppresses only ochre (UAA) markers and causes the insertion of tyrosine (GILMORE, STEWART and SHERMAN 1971).

Omnipotent suppressors: The omnipotent suppressors used in this study were induced in strain SL183-21C (*α cyc1-76 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 his5-2 lys2-1 leu2-1 can1-132*) by UV irradiation. Revertants were selected that grew on synthetic media containing all the growth requirements except methionine and leucine. The suppressors obtained acted on the ochre marker *leu2-1*, the amber marker *met8-1* and, in many cases, on the amber marker *tyr7-1*. The suppressors were recessive and fell into two complementation groups. Since *sup45* and *sup35* strains were not available for complementation tests, we mapped our suppressors to see if they were located near *sup45* and *sup35*. A suppressor from the first complementation group mapped 20 cM from *lys2-1*, as does HAWTHORNE and MORTIMER's (1968) *sup45*. Thus, we assumed that these suppressors are alleles of *sup45* and have named them *sup45-2*, *sup45-3*, etc. The wild-type allele of this locus is called *SUP45*+, and an intragenic revertant of *sup45-2* has been called *SUP45-2-a*+. A suppressor from the other complementation group mapped 8 cM from *hom2*, as does HAWTHORNE and MORTIMER's (1968) *sup35*. We have named these suppressors *sup35-2*, *sup35-3*, etc., since the mapping data suggest that they are alleles of *sup35*. The wild-type allele of this locus is called *SUP35*+ or +.

Antisuppressors: Antisuppressors were selected in strains L-475 and SL428-1C, both with the genotype *α cyc1-76 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys2-1 can1-132 sup45-2*, and in strain SL428-13D with the genotype **a** *cyc1-76 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1 can1-132 sup45-2*. From 10^6 to 10^7 cells/plate were spread on synthetic glucose media containing 60 mg/l canavanine, on hypertonic media or on nutrient media containing 100 mg/l paromomycin (gift of H. E. MACHAMER, Warner-Lambert/Parke-Davis, Detroit, MI). Some plates were exposed to 1200 ergs/mm^2 of UV light. Revertants selected on these plates were then subjected to additional tests as described in RESULTS. Eventually, 6 allelic antisuppressors that reduce the efficiency of *sup45* were recovered. These antisuppressors have been named *asu9-1*, *asu9-2*, *asu9-3*, *asu9-4*, *asu9-5* and *asu9-6*. The wild-type allele of this locus is referred to as *ASU9*+, *ASU*+ or + .The phenotypes of these antisuppressor strains are described in Table 2.

Genetic methods

Standard yeast-genetic procedures of crossing, sporulation and tetrad analysis were used to construct appropriate strains and analyze gene segregations. Replica plating was accomplished by the use of cell suspensions and a replicator having inoculating rods.

TABLE 2

Phenotypes of parent and antisuppressor strains

Strain	Partial genotype	Isolated from	Mutagen	Growth on —met	Growth on —tyr	Growth on —leu
SL183-21C	+ +			—	—	—
SL428-1C	*sup45-2* +			+	+	+
L-477	*sup45-2 asu9-1*	SL428-1C	spontaneous	±	—	±
L-479	*sup45-2 asu9-2*	SL428-1C	spontaneous	—	—	—
L-493	*sup45-2 asu9-3*	SL428-1C	1250 ergs/mm^2 UV	—	—	—
L-497	*sup45-2 asu9-4*	SL428-1C	spontaneous	±	—	±
L-475	*sup45-2* +			+	+	+
L-500	*sup45-2 asu9-5*	L-475	1200 ergs/mm^2 UV	—	—	—
SL428-13D	*sup45-2* +			+	+	+
L-501	*sup45-2 asu9-6*	SL428-13D	1200 ergs/mm^2 UV	—	—	—

All strains carry the amber mutations *met8-1* and *tyr7-1* and the ochre mutation *leu2-1*.
Growth was estimated by comparing spots on plates made by inoculations with suspensions of cells. + indicates good growth by 2 days; ± indicates some growth by 4 days; — indicates no sign of growth by 6 days.

Nutritional markers were scored by growth on synthetic glucose medium containing 0.67% (w/v) Bacto-yeast nitrogen base (without amino acids), 2% (w/v) Bactoagar, and appropriate amino acids. Canavanine resistance was scored on plates (+CAN) of synthetic glucose media lacking arginine but containing all required amino acids, plus 60 mg/l canavanine sulfate (a poisonous arginine analog). The segregation of the *sup* genes was scored by suppression of appropriate homozygous amber and ochre markers. Sensitivity to hypertonic media was scored on plates containing nutrient medium (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, 2% Bactoagar) supplemented with 15% ethylene glycol or 30% dextrose. Sensitivity to the aminoglycoside antibiotic, paromomycin, was scored on plates containing nutrient medium supplemented with 1 mg/ml or 5 mg/ml paromomycin.

Antisuppressors were usually scored by their effect in lowering suppression efficiciency in pedigrees that were homozygous for an omnipotent suppressor. In crosses where a suppressor was not present, or was present in a heterozygous condition, backcrosses were performed to distinguish between *asu9 SUP+*, *asu9 sup* and *ASU9+ SUP+* segregants. In some crosses, *asu9* was scored directly by its characteristic inhibition of growth on media supplemented with 5 mg/ml paromomycin.

Efficiency of suppression

The efficiency of suppression was estimated by measuring the growth rate of suppressed strains on media where suppressor activity is required for growth. In addition, in some pedigrees, the efficiency of suppression of the amber mutation *cycl-76* (STEWART and SHERMAN 1973) or the ochre mutation *cycl-72* STEWART *et al.*, unpublished; see SHERMAN and STEWART 1974) in the iso-1-cytochrome *c* gene was determined by estimating the level of iso-1-cytochrome *c* in suppressed mutants. This was accomplished by low-temperature (—190°) spectroscopy of whole cells (SHERMAN and SLONIMSKY 1964). A more precise measure of efficiency of suppression can be obtained with the *cyc1* mutants than with the nutritional mutants since the levels of suppressed iso-1-cytochrome *c* can be determined directly instead of being inferred from growth rate. Indeed, suppressors that have the same effect on auxotrophic markers can be distinguished by their various effects on the amber marker *cyc1-179* (LIEBMAN, SHERMAN and STEWART 1976).

RESULTS

Selection for antisuppressors

Antisuppressors were selected that reduce the efficiency of the omnipotent, recessive suppressor, *sup45–2*. The origin of the *sup45–2* allele used in this study is described in MATERIALS AND METHODS. Three different rationales, described separately below, were employed to obtain antisuppressors in strains SL428–1C, SL428–13D and L-475. All of these parent strains contain *sup45–2* and the suppressible amber mutations, *met8–1*, *tyr 7–1* and *can1–132*, as well as the suppressible ochre mutation, *leu2–1*.

Canavanine resistance selection: The parent strains cannot grow on media containing canavanine, since the *can1–132* allele, which confers resistance to canavanine, is suppressed by *sup45–2*. Mutations that reduce the efficiency of *sup45–2* and thereby eliminate suppression of *can1–132* render the strains resistant to canavanine. Thus, SL428–1C and SL428–13D were plated on media containing canavanine, and resistant colonies were picked as presumptive antisuppressors.

Osmotic resistance selection: Many dominant amber- and ochre-specific suppressors have been previously shown to cause poor growth on osmotic media (SINGH 1977). Although the mechanism is not understood, the inhibition of growth on hypertonic media caused by these suppressors is correlated with their

efficiencies of suppression, *i.e.*, the sensitivity is lost when suppression efficiency is reduced.

In the present work, we found that the omnipotent suppressor, *sup45–2*, is also sensitive to growth on hypertonic media. The analysis of 15 tetrads from a diploid heterozygous for *sup45–2* showed that the inability to grow on the hypertonic medium always segregated with *sup45–2*. Thus, antisuppressors that reduce the activity of *sup45–2* were selected as revertants of SL428–1C, SL428-13D and L-475 able to grow on high osmotic media.

Paromomycin resistance selection: It has recently been shown that the growth of *sup45*-containing strains, but not wild-type strains, is inhibited by low doses of the aminoglycoside antibiotic, paromomycin (PALMER, WILHELM and SHERMAN, personal communication). Thus, antisuppressors to *sup45–2* were selected as paromomycin-resistant revertants of the *sup45*-bearing strains, L-475 and SL428–13D.

Check for reduced suppression

Only some of the 625 mutants selected on the canavanine, high osmotic and paromomycin media contain antisuppressors, since a variety of other mutations can also cause resistance to these agents. Therefore, resistant mutants were tested for antisuppressor activity by spotting on omission media where suppressor activity was required for growth. One hundred of these mutants exhibited reduced suppressor activity, as judged by reduced growth on the omission media (see Table 3). Only these mutants were considered further.

A spore test to distinguish between antisuppressors at unlinked loci and intragenic suppressor revertants

Mutants with reduced suppressor activity may be either unlinked antisuppressors or revertants at the suppressor locus. To distinguish between these possibilities, we determined if the fully active suppressor was still present in the mutant. Mutants with reduced suppressor activity were crossed with testers con-

TABLE 3

Screening for antisuppresssors

Parent strain	Phenotype selected	Number of resistant colonies picked	Number* tested in spore test	Number yielding intergenic revertants	Antisuppressors studied further
SL428–1C	Canavanine resistance	150	32	7	*asu9–1*; *asu9–4*
SL428–13D	Canavanine resistance	30	6	0	
SL428–1C	Osmotic resistance	210	39	9	*asu9–2*; *asu9–3*
SL428–13D	Osmotic resistance	120	13	1	
L-475	Osmotic resistance	30	0	0	
L-475	Paromomycin resistance	30	6	5	*asu9–5*
SL428–13D	Paromomycin resistance	55	4	3	*asu9–6*

* The strains that were put through the spore test were chosen for their reduced efficiency of suppression. The spore test to distinguish between antisuppressors at unlinked loci and intragenic suppressor revertants is described in the text.

taining the suppressible markers *tyr7–1*, *leu2–1* and *met8–1*, but not *sup45–2*. The diploids were sporulated and the spore mixtures of whole asci and corresponding haploid mutants (either antisuppressors or revertants) were respectively spotted on media that require suppression for growth. In 75 cases, the spore suspensions grew no better than their antisuppressor or revertant parent, indicating that the fully active *sup45–2* allele was not present in any of the segregants. This implies that these mutations that have lost suppressor activity are reversions at, or very near, the suppressor locus. One such strain, SL-471, was dissected (see Table 4) and, indeed, *sup45–2* was not recovered in any segre-

TABLE 4

Six antisuppressor mutations segregate independently from sup45

Cross*	Genotype	Number of tetrads†		
		sup45 + / *sup45* + / + *asu9*‡ / + *asu9*‡	*sup45* *asu9*§ / *sup45* *asu9*§ / + + / + +	*sup45* + / *sup45* *asu9* / + + / + *asu9*
SL-428	$\frac{sup45\text{–}2}{+}$	15	0	0
SL-471	$\frac{SUP45\text{–}2\text{–}a^{+}}{+}$	0	5	0
SL-434	$\frac{sup45\text{–}2}{+}\ \frac{asu9\text{–}1}{+}$	4	6	17
SL-437	$\frac{sup45\text{–}2}{+}\ \frac{asu9\text{–}2}{+}$	1	3	5
SL-468	$\frac{sup45\text{–}2}{+}\ \frac{asu9\text{–}3}{+}$	2	1	4
SL-476	$\frac{sup45\text{–}2}{+}\ \frac{asu9\text{–}4}{+}$	2	2	7
SL-488	$\frac{sup45\text{–}2}{+}\ \frac{asu9\text{–}5}{+}$	0	0	4
SL-491	$\frac{sup45\text{–}2}{+}\ \frac{asu9\text{–}6}{+}$	4	0	3

* SL-428 is a control with the genotype shown. The spore test indicated that SL-471 contains an intragenic revertant at the *sup45* locus; its presumed genotype is shown. The spore test indicated that SL-434, SL-437, SL-468, SL-476, SL-488 and SL-491 contained antisuppressors unlinked to *sup45–2*; their presumed genotypes are shown. All diploids are homozygous for the amber markers *met8–1* and *tyr7–1* and for the ochre marker *leu2–1*.

† Segregants were scored by comparing the growth of spots made by inoculations with suspensions of cells on —met, —tyr and —leu plates. The genotypes of the tetrads were deduced by scoring the segregants as follows and by assuming a 2:2 segregation for *sup45* and *asu9*. Strains with the genotype *sup45* + grew well on all three types of media within two days; + +, + *asu9* and some *sup45 asu9* strains did not grow at all on any of the media by 6 days, while some *sup45 asu9* strains grew slightly on —met and —leu media, but not on —tyr medium.

‡ In the SL-428 pedigree, the genotypes of these segregants should read + +.

§ In the SL-471 pedigree, the genotype of these segregants should read *SUP45–2–a*+.

gants among the five tetrads examined. The spores of 25 other strains exhibited restored suppressor activity compared with the corresponding revertant parent, indicating that the fully active *sup45–2* suppressor was segregating in these progeny. Thus, these mutants were considered to contain antisuppressors unlinked to *sup45–2*. When six of these strains were dissected, *sup45–2* segregated independently from each of the antisuppressors (see Table 4). These six antisuppressors have been studied further; their phenotypic properties were summarized in Table 2.

Are antisuppressor mutations dominant or recessive?

Diploids were constructed to be homozygous for the *sup45–2* suppressor and the suppressible markers *met8–1*, *tyr7–1* and *leu2–1*. Some of these diploids were heterozygous (*asuX*/+) and some were homozygous (+/+ and *asuX*/*asuX*) for each of the six independently isolated antisuppressors. The growth of these strains on solid omission media was then compared. On media lacking methionine or leucine, the *asuX*/+ and +/+ strains both grew at the same rate in a spot test. In contrast, on media lacking tyrosine the *asuX*/+ strains grew more slowly than +/+ strains, but faster than *asuX*/*asuX* strains. Thus, the antisuppressors are recessive when scored on the basis of suppression of *met8–1* or *leu2–1*, but semidominant when scored on the basis of suppression of *tyr7–1*.

Mendelian inheritance

Diploids that were homozygous for *sup45–2* and heterozygous for each of the six antisuppressors, respectively, were sporulated and dissected. Among the 15 (*asu9–1*), six (*asu9–2*), five (*asu9–3*), 14 (*asu9–4*), seven (*asu9–5*) and six (*asu9–6*) tetrads examined, the antisuppressor always segregated 2:2.

Complementation groups

Diploids were constructed that were homozygous for *sup45–2* and heterozygous for each of two independently isolated antisuppressors. Since each antisuppressor is recessive when scored on -met or -leu media, suppressor activity should be restored in these diploids if the antisuppressors are at distinct loci. The data from these complementation tests (Table 5) indicate that all the anti-

TABLE 5

Summary of complementation data

Antisuppressor No.	1	2	3	4	5	6
1	—	—	—	—	—	—
2		—	—	—	—	—
3			NA	—	—	—
4				—	—	—
5					—	—
6						—

Antisuppressors numbered 1 to 6 correspond to allele assignments *asu9–1* to *asu9–6*. All diploids are homozygous for *sup45–2*.
— indicates that suppressor activity is not restored in the diploid.
NA signifies that data are not available.

suppressors are at a single locus, since suppressor activity is never restored in the diploid. This conclusion was further verified by sporulating and dissecting diploids from the pairwise crosses *asu9–1* × *asu9–5*, *asu9–2* × *asu9–4*, *asu9–2* × *asu9–3* and *asu9–1* × *asu9–6*. Among the 11, 11, three and six tetrads examined, respectively, the suppressor phenotype was not recovered in any of the segregants. This indicates that the two heterozygous antisuppressors in each of the crosses must be alleles. The six allelic antisuppressors have been named *asu9–1*, *asu9–2*, *asu9–3*, *asu9–4*, *asu9–5* and *asu9–6*.

Omnipotent suppressors are affected by asu9

The *asu9* alleles were all isolated as mutations that reduce the efficiency of a single omnipotent suppressor, *sup45–2*. HAWTHORNE and LEUPOLD (1974) previously showed that different alleles at the same omnipotent suppressor locus can exhibit different properties. Thus, it was of interest to examine the effect of *asu9* on a different *sup45* allele. Accordingly, *asu9–1* was outcrossed from *sup45–2* so that it could be tested for its action on *sup45–3*. The *asu9–1 SUP45*+ strain was obtained from a nonparental ditype tetrad of the SL-434 pedigree (see Table 4). Barring gene conversion, the genotype of the *asu9–1 SUP45*+ segregant chosen could be unambiguously inferred from the tetrad data. However, since the phenotype of antisuppressor-bearing segregants (*asu9–1 SUP45*+) on omission media is identical to that of wild type (*ASU9*+ *SUP45*+) strains, the presence of the antisuppressor was verified by a backcross to the original *sup45–2*-bearing strain (SL428–1C). As expected, the antisuppressor segregated in the progeny and, when coupled with *sup45–2*, reduced suppression efficiency. Similarly, when the *asu9–1 SUP45*+ strain was crossed with an *ASU9*+ *sup45–3* strain, the antisuppressor reduced the efficiency of *sup45–3* in the segregants (*asu9–1 sup45–3*).

The action of *asu9* on the omnipotent suppressor locus, *sup35–2*, was examined next. Strains carrying three independent alleles of *asu9* were crossed with a *sup35–2* strain. In each case, *asu9* segregated independently from *sup35–2* and reduced the efficiency of *sup35–2* suppression. Thus, *asu9–1* is not specific for the *sup45–2* allele against which it was isolated, but acts on other alleles of *sup45*, as well as on *sup35*.

Amber and ochre suppressors are not affected by asu9

In order to test the action of *asu9–1* on amber suppressors, a diploid heterozygous for *asu9–1* and the tyrosine-inserting amber suppressor, *SUP7-a*, was constructed. The segregation and efficiency of *SUP7-a* was determined in the segregants by testing for suppression of the homozygous amber nutritional markers, *met8–1*, *tyr7–1*, *trp1–1*, *ade3–26* and *ilv1–1*. In addition, the homozygous amber marker, *cyc1–76*, permitted the efficiency of suppression in the segregants to be estimated from the level of iso-1-cytochrome *c*. In all of the 14 tetrads examined, there was a clear 2:2 segregation for an efficient amber suppressor. The suppressors were judged to be efficient because segregants bearing them grew as well on omission media and contained as much iso-1-cyto-

chrome *c* (approximately 75% of the normal level) as their suppressed parent. This indicates that either *asu9–1* does not act on *SUP7-a* to reduce its efficiency or *asu9* is very lightly linked to *SUP7-a*.

In similar experiments where *asu9–2* was tested against *SUP7-a* and *asu9–1* was tested against the leucine-inserting amber suppressor, *SUP52-a*, there was no indication of antisuppressor action on the amber suppressors among the 12 and 14 tetrads examined, respectively.

In order to test the action of *asu9–1* on ochre suppressors, the progeny from a diploid heterozygous for *asu9–1* and *SUP7–o* were examined. The segregation of *asu9–1* was scored directly as paromomycin-sensitive segregants (see Pleiotropic effects of *asu9* and MATERIALS AND METHODS), while the segregation of the suppressor was scored by suppression of homozygous ochre markers. The presence of *asu9–1* did not alter the ability of *SUP7-o* to suppress any of the tested ochre markers, including *his5–2*, *leu2–1*, *lys1–1*, *can1–100* and *cyc1–72*.

Absence of centromere linkage

In order to determine whether the *asu9* gene is centromere linked, a diploid strain was constructed to be homozygous for the suppressible markers *met8–1*, *leu2–1*, and *tyr7–1* and for the omnipotent suppressor *sup35–2*. In addition, the strain was heterozygous for the centromere marker, *ura3* (5.1 cM from centromere, MORTIMER and HAWTHORNE 1966), and for *asu9–1*. The strain was sporulated and segregants were scored for the presence of *ura3* and *asu9–1*. Among the 14 tetrads examined, 12 were tetratypes, indicating that *asu9–1* is not centromere linked (less than 67% tetratypes would indicate centromere linkage).

Pleiotropic effects of asu9

Segregants from crosses that were heterozygous for the antisuppressor (*asu9*/+) and either homozygous (+/+) or heterozygous (*sup45–2*/+) for the suppressor, where analyzed for growth rate and sensitivity to paromomycin. The genotypes of these segregants were scored unambiguously by performing appropriate back crosses.

When separated from *sup45–2*, the *asu9* mutations do not exhibit any suppressor activity; however, relative to wild type, a reduced growth rate at 25° and increased sensitivity to paromomycin do segregate with *asu9*. It has previously been noted that some recessive omnipotent suppressors also cause poor growth (SMIRNOV *et al.* 1974; GERLACH 1975; LIEBMAN, SHERMAN and STEWART 1976) and an increased sensitivity to paromomycin (PALMER, WILHELM and SHERMAN, private communication); the *sup45–2* suppressor used in this study was found to exhibit both of these characteristics.

The *sup45–2* mutation caused more sensitivity to paromomycin than did the *asu9* mutation; whereas, dominant tRNA-type suppressors did not cause any paromomycin sensitivity. A 1 mg/ml dose of paromomycin in solid glucose-containing medium was capable of completely inhibiting the growth of *sup45–2* strains, but not of *asu9–1* strains. A 5 mg/ml dose of the antibiotic prevented any growth of *asu9–1* strains, but permitted wild-type strains to grow slowly.

The sensitivity of *asu9–1* to paromomycin is particularly curious since *asu9–1* can reverse the paromomycin sensitivity of *sup45–2*. Indeed, the antisuppressor alleles, *asu9–5* and *asu9–6*, were selected as paromomycin-resistant revertants of *sup45–2* strains on medium containing 1 mg/ml paromomycin. Growth of the *sup45 asu9* double-mutants is inhibited by 5 mg/ml, but not 1 mg/ml, paromomycin, although these strains are generally slightly more drug resistant than *asu9* strains that do not contain *sup45*.

DISCUSSION

Current evidence suggests that the omnipotent suppressors, *sup45* and *sup35*, cause altered ribosomes that lead to translational errors, including the misreading of nonsense codons. Indeed, the small ribosomal subunit from *sup45* strains has recently been shown to cause misreading in a cell-free poly(*u*)-directed protein synthesis system (MASUREKAR, PALMER, WILHELM and SHERMAN, personal communication). In the present paper, six antisuppressor mutations that reduce the efficiency of the omnipotent suppressor, *sup45*, have been isolated and shown to fall within a single Mendelian gene, named *asu9*. In the presence of some *asu9* alleles, *sup45* entirely loses its ability to suppress three nonsense mutations with a reduced efficiency. The activity of another omnipotent suppressor, *sup35*, is also reduced by *asu9*, but *asu9* does not affect several amber- or ochre-specific suppressors that presumably result from altered tRNAs. The phenotypic properties of *asu9*-bearing strains suggest that the antisuppressor reduces translational errors caused by omnipotent suppressors.

The *asu9* gene is not centromere linked, nor is it linked to either of the omnipotent suppressors upon which it acts. Allelism tests have not been performed between *asu9* and the eight previously isolated recessive antisuppressors (MCCREADY and COX 1973) that reduce the activity of ochre suppressors. However, since *asu9* does not act on the ochre suppressor *SUP7-o*, it probably represents a new genetic locus.

The growth of some suppressor and antisuppressor strains is inhibited by paromomycin, a drug that has been shown to cause translational misreading in yeast (SINGH, URSIC and DAVIES 1979; PALMER, WILHELM and SHERMAN 1979). A paromomycin concentration of 1 mg/ml completely inhibits the growth of *sup45* strains, while 5 mg/ml is required to inhibit *asu9* or *sup45 asu9* double mutants. In contrast, growth of wild-type and *sup35* strains is unaffected by the drug at either concentration. It has been suggested that the paromomycin inhibition of *sup45* strains results from an inordinate amount of misreading, since paromomycin was found to enhance the *in vitro* misreading effected by *sup45* ribosomes (MASUREKAR *et al.*, personal communication). According to this hypothesis, we would expect the *asu9* antisuppressor, which reduces these translational errors, also to reduce the *sup45*-induced paromomycin sensitivity. Indeed, we find *sup45 asu9* double mutants to be more resistant to paromomycin than are *sup45* mutants. Paradoxically, *asu9* by itself, without *sup45*, also causes sensitivity to paromomycin.

The mechanism of action of the *asu9* mutation is unknown. Numerous possibilities involving all facets of the translational apparatus can be envisaged; here, we speculate on one possibility. In this model, the *asu9* mutation alters a ribosomal protein, either directly or indirectly. To explain the paromomycin sensitivity associated with *asu9*, we propose that these *asu9*-altered ribosomes would translate very slowly, if at all, in the presence of paromomycin, leading to a cessation of growth. Similarly, the *asu9* alteration might also decrease the translation rate of *sup45* error-prone ribosomes, whether or not paromomycin is present. This decreased translation rate may allow the correct tRNA to have time to bind to the ribosome in successful competition with incorrect tRNAs and could explain how *asu9* restricts misreading.

Antisuppressors similar to *asu9* have been uncovered in other fungi. Class A antisuppressors in *Schizosaccharomyces pombe* act on omnipotent suppressors, but not on tRNA-type suppressors. One of these antisuppressors is resistant to the ribosomal inhibitor, cycloheximide (THURIAUX *et al.* 1975). In *Podospora anserina*, antisuppressors AS_1 and AS_2 were selected as mutations affecting ribosomal-like suppressors (PICARD-BENNOUN 1976). Unlike *asu9*, the AS_1 and AS_2 antisuppressors do act on tRNA-type suppressors.

Genetic evidence presented here suggests that the *asu9* mutation causes an alteration in the translational apparatus. A biochemical approach will now be necessary to determine the molecular mechanisms involved.

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