Allosuppressors that Enhance the Efficiency of Omnipotent Suppressors in *Saccharomyces cerevisiae*

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ABSTRACT

Two recessive Mendelian-allosuppressors have been isolated and have been shown to enhance the efficiency of omnipotent suppressors thought to be translational ambiguity mutations. These allosuppressors are unlinked to each other or to the omnipotent suppressors on which they act. They also increase the efficiency of the serine-inserting UAA-suppressor, *SUP16*. One allosuppressor is allelic or tightly linked to the previously isolated *sal2*. Another allosuppressor, called *sal6*, represents a new locus, unlinked to the previously isolated *sal1-sal5* that enhance the efficiency of the UAA-suppressors. When present singly in the absence of suppressors or other modifiers the *sal2* and *sal6* mutations do not have suppressor activity. However, when *sal2* and *sal6* are combined together in a haploid cell they do suppress weakly. In addition *sal2* becomes a weak suppressor in the presence of the [η^+] modifying factor.

MANY nonsense suppressors have been described in *Saccharomyces cerevisiae* (HAWTHORNE and LEUPOLD 1974; SHERMAN, ONO and STEWART 1979; SHERMAN 1982). The best studied of these suppressors are codon-specific, suppressing UAA, UAG or UGA nonsense codons. Most of these suppressors are dominant or semidominant, and several have been definitively shown to arise from mutations in the anticodon of tRNA genes (PIPER *et al.* 1976; GOODMAN, OLSON and HALL 1977; PIPER 1978; ETCHEVERRY, COLBY and GUTHRIE 1979; BROACH, FRIEDMAN and SHERMAN 1981; LIEBMAN *et al.* 1984). Another class of suppressors, called omnipotent suppressors, is codon-nonspecific and causes translational errors that lead to the misreading of all three nonsense codons (INGE-VECHTOMOV and ANDRIANOVA 1970; HAWTHORNE and LEUPOLD 1974; GERLACH 1975; ONO, STEWART and SHERMAN 1981; J. ALL-ROBYN, E. GRIFFIN and S. LIEBMAN, unpublished results). Current evidence suggests that the translational inaccuracy caused by these suppressors results from altered ribosomes (SMIRNOV *et al.* 1973, 1974, 1976, 1978; SURGUCHOV *et al.* 1980, 1981; ISHIGURO *et al.* 1981; MASUREKAR *et al.* 1981; EUSTICE *et al.* 1986). Additional genes involved in protein synthesis can be identified by selecting for mutations that modify the efficiency of suppressors. A variety of such modifiers that either enhance or reduce suppression efficiency have been isolated in both prokaryotes (COLBY, SCHEDL and GUTHRIE 1976; PIEPERSBERG *et al.* 1980; SULLIVAN and BOCK 1985) and eukaryotes (MCCREADY and COX 1973; HAWTHORNE and LEUPOLD 1974; THURIAUX *et al.* 1975; LATEN, GORMAN and BOCK 1978; HOPPER *et al.* 1981; RANDSHOLT *et al.* 1982; NURSE and THURIAUX 1984; DEQUARD-CHABLAT 1985).

In yeast, three Mendelian antisuppressor genes, *asu9* (LIEBMAN and CAVENAGH 1980), *ASU10* (LIEBMAN, CAVENAGH and BENNETT 1980) and *asu11* (ISHIGURO 1981), that reduce the efficiency of omnipotent suppressors have been described. The *asu9* and *asu11* mutations were each shown to cause altered ribosomes (LIEBMAN and CAVENAGH 1981; ISHIGURO 1981). In addition, the non-Mendelian [η^+] factor causes lethality of certain omnipotent suppressor strains (LIEBMAN and ALL-ROBYN 1984) and increases the efficiency of another omnipotent suppressor (J. ALL-ROBYN, E. GRIFFIN and S. LIEBMAN, unpublished results). Five allosuppressor genes, *sal1-sal5*, that increase the efficiency of the serine-inserting UAA-suppressor *SUP16*, have been described (COX 1977).

In this paper, we describe the isolation and genetic characterization of two allosuppressor genes, *sal2* and *sal6*, capable of increasing the efficiency of various omnipotent suppressors and the UAA-suppressor *SUP16*.

MATERIALS AND METHODS

Suppressible markers: The properties of the suppressible nutritional or resistance markers used here are described in LIEBMAN, SHERMAN and STEWART (1976). The *met8-1*, *aro7-1*, *trp1-1*, *ade3-26*, *ilv1-1* and *cyc1-76* alleles are amber (UAG) mutations, while the *leu2-1*, *his5-2*, *lys1-1*, *lys2-1* and *ade2-1* alleles are ochre (UAA) mutations.

Omnipotent suppressors: The phenotypes of the recessive omnipotent suppressor strains used here are described in LIEBMAN and CAVENAGH (1980) and SONG and LIEBMAN (1985). The *sup45-2* allele suppresses the ochre marker *leu2-1*, the amber marker *met8-1*, and in many cases, the amber

markers, *aro7-1* and *trp1-1*. The *sup35-4* allele confers temperature-sensitivity for growth at 37° and suppresses the ochre marker *leu2-1* and the amber marker *met8-1* at the permissive temperature. Two dominant omnipotent suppressors, *SUP38-1* and *SUP39-1*, used here have recently been isolated (J. ALL-ROBYN, E. GRIFFIN and S. LIEBMAN, unpublished results) and suppress the ochre marker *leu2-1* and the amber marker *met8-1*.

Antisuppressors: The phenotypes of antisuppressor strains used here are described in LIEBMAN and CAVENAGH (1980). The *asu9-1* gene reduces the suppression efficiency of the omnipotent suppressor, *sup45-2*.

Allosuppressors: Allosuppressors were selected in strains, SL436-3D, with the genotype a *sup45-2 asu9-1 cyc1-76 met8-1 aro7-1 trp1-1 ade3-26 ilv1-1 leu2-1 lys1-1 can1* and L-477, with the genotype α *sup45-2 asu9-1 cyc1-76 met8-1 aro7-1 trp1-1 ade3-26 ilv1-1 leu2-1 lys2-1 can1*. From 10^6 to 10^7 cells/plate were spread on synthetic glucose medium lacking methionine and leucine, but containing all other required amino acids. Some plates were exposed to 500 ergs/mm^2 of UV light. Revertants selected on these plates were then subjected to additional tests as described in RESULTS. Eventually, two recessive allosuppressor genes, *sal2* and *sal6*, that restored suppression efficiency in *asu9-1 sup45-2* strains were recovered. The wild type alleles of these loci are referred to as *SAL*$^+$. The phenotypes of these allosuppressor strains are described in Table 1. The genes *sal1-sal5* were previously isolated as allosuppressors that increased the efficiency of the serine-inserting UAA-suppressor *SUP16* (COX 1977) and were obtained from M. TUITE. One of the *sal2* alleles isolated in this study is referred to as *sal2-B* and that obtained from M. TUITE is called *sal2-A*.

Genetic methods: Standard yeast genetic procedures of crossing, sporulation and tetrad analysis were used to construct appropriate strains and analyze gene segregations (SHERMAN and LAWRENCE 1974). Replica plating was accomplished by the use of cell suspensions and a replicator having inoculating rods. Standard media (SHERMAN, FINK and HICKS 1982) were used for scoring various markers. Nutritional markers were scored by growth on synthetic glucose media containing 0.67% (w/v) Bacto-yeast nitrogen base (without amino acids), 2% (w/v) Bacto-agar and appropriate amino acids. Sensitivity to the aminoglycoside antibiotic, paromomycin, was scored on plates containing nutrient medium supplemented with 0.1, 0.2, 0.5, 1 or 5 mg/ml paromomycin. Hypersensitivity to the antibiotic trichodermin was scored on plates containing nutrient medium supplemented with 1 μg/ml trichodermin.

The segregation of the suppressor genes was scored by analyzing the suppression of appropriate homozygous amber and ochre markers. Antisuppressors (*asu*) and allosuppressors (*sal*) were usually scored by their effect on decreasing or increasing suppression efficiency, respectively, in pedigrees that were homozygous for an affected suppressor. In crosses where an appropriate suppressor was not present, or was present in a heterozygous condition, backcrosses were performed to score for the *asu* or *sal* genes. In some crosses, *asu9* was scored directly by its characteristic inhibition of growth on media supplemented with 5 mg/ml paromomycin or 1 μg/ml trichodermin.

Efficiency of suppression: The efficiency of suppression was determined by the different spectra of markers suppressed, and by the level of growth that the suppressor permitted on appropriate omission media in spite of the presence of suppressible auxotrophic markers. A single definitive value cannot be given, since the efficiency of suppression varied depending on the auxotrophic markers scored. In addition, the suppression efficiency varied somewhat in different strains having the same pertinent genotypes. The strain variation was probably due to numerous modifying genes, which could affect either true suppressor efficiency or the expression of auxotrophic genes. Thus, the suppression efficiency was measured by comparing the growth of spots on plates made by inoculations with suspensions of cells. In this way, the efficiency of suppression in large numbers of segregants could be easily scored. At the same time, the efficiency of suppression of many different auxotrophic markers in each segregant could be examined. In addition, to get some quantitative measure of the efficiency of suppression the growth rate was carefully determined in a few representative strains on −Leu media. These strains were grown in liquid synthetic media with and without leucine. Samples were withdrawn at various times and the optical density determined at A_{600}. The ratio of the doubling time in −Leu media to that in the synthetic complete media was used as a measure of the efficiency of suppression.

RESULTS

Selection for allosuppressors: The omnipotent suppressor, *sup45-2*, is capable of suppressing the amber mutations, *met8-1*, *aro7-1*, and *trp1-1* as well as the ochre mutation *leu2-1*. In the presence of the antisuppressor mutation, *asu9-1*, the efficiency of *sup45-2* is greatly reduced (see Table 1). Thus, in order to obtain mutants which either enhance suppressor activity or reduce antisuppressor activity, revertants of *asu9-1 sup45-2* strains with restored suppressor activity were selected. Strains SL436-3D (a *sup45-2 asu9-1 cyc1-76 met8-1 aro7-1 trp1-1 ade3-26 ilv1-1 leu2-1 lys1-1 can1*) and L-477 (α *sup45-2 asu9-1 cyc1-76 met8-1 aro7-1 trp1-1 ade3-26 ilv1-1 leu2-1 lys2-1 can1*) were plated on medium lacking methionine and leucine. These strains grow very poorly on this medium because *asu9-1* prevents *sup45-2* from suppressing *met8-1* and *leu2-1*. Revertants capable of good growth on this medium were picked as presumptive suppressor enhancers.

Scan for recessive allosuppressors: Recessive revertants were sought because these could easily be placed into complementation groups. Furthermore, the recessive revertants are not likely to be intragenic revertants of the *asu9-1* mutation since these would be expected to be dominant. Thus they would be more likely to represent new loci.

Each of the 93 revertants isolated from SL436-3D or L-477 was crossed respectively with L-477 or SL436-3D. If the suppressor-activating mutations are not in the *sup45* or *asu9* loci, these diploids would be homozygous for *sup45-2* and *asu9-1* and heterozygous for the suppressor-activating mutation. In addition, each of the diploids is homozygous for the suppressible markers, *met8-1*, *leu2-1*, *aro7-1* and *trp1-1*. Diploids were isolated on medium lacking lysine and the suppressor activity present in each of these diploids was compared to the suppressor activity in the correspond-

TABLE 1

Phenotypes of omnipotent suppressor, antisuppressor and allosuppressor strains

Partial genotype	Complete glucose		Complete glucose + paromomycin, mg/ml					Tricho-dermin, 1 μg/ml	Synthetic complete minus						Relative growth rate on −Leu[c]
	30°	17°	0.1	0.2	0.5	1.0	5.0		Met	Leu	Trp	Tyr	Ilv	Ade	
SAL⁺ ASU⁺ SUP⁺	++	+	++	++	++	++	+	+	−[a]	−	−	−	−	−	0
asu9-1 [η⁻]	++	+	++	++	+	±	−	−	−[a]	−	−	−	−	−	
sup45-2 [η⁻]	++	+	+	−	−	−	−	+	++	++	±	±	−	−	0.45
asu9-1 sup45-2 [η⁻]	++	±	++	+	±	−	−	−	±	±	−	−	−	−	0.04
sal6-1 asu9-1 sup45-2 [η⁻]	++	−	+	±	−	−	−	−	++	++	±	−	−	−	
sal6-1 sup45-2 [η⁻]	++	−	−	−	−	−	−	+	++	++	++	++	±	−	0.79
sal6-1 [η⁻] or [η⁺]	++	+	++	++	++	++	+	+	−[a]	−	−	−	−	−	
sal2-B asu9-1 sup45-2 [η⁻]	++	±	++	+	−	−	−	−	++	++	±	−	±	−	
sal2-B sup45-2 [η⁻]	++	±	±	−	−	−	−	+	++	++	++	++	±	±	0.93
sal2-B [η⁻]	++	+	++	++	++	++	+	+	−[a]	−	−	−	−	−	
sup35-4[b] [η⁻]	−	++	++	+	−	−	−	+	++	++	−	−	−	−	
sal6-1 sup35-4[b] [η⁻]	−	++	+	±	−	−	−	+	++	++	±	±	−	−	
sal2-B sup35-4[b] [η⁻]	−	++	+	±	−	−	−	+	++	++	±	±	−	−	
sal6-1 asu9-1 [η⁻]	++	+	++	++	+	±	−	−	−[a]	−	−	−	−	−	
sal2-B asu9-1 [η⁻]	++	+	++	++	+	±	−	−	−[a]	−	−	−	−	−	
sal6-1 sal2-B [η⁻]	++	+	++	++	++	+	−	+	−[a]	±	−	−	−	−	
sal2-B [η⁺]	++	+	++	++	++	++	+	+	−[a]	±	−	−	−	−	
SUP38-1 [η⁺]	++	+	+	−	−	−	−	+	++	++	−	−	−	−	
sal6-1 SUP38-1 [η⁺]	++	+	−	−	−	−	−	+	++	++	−	±	−	−	
sal2-B SUP38-1 [η⁺]	++	+	−	−	−	−	−	+	++	++	++	++	+	++	
SUP38-1 [η⁻]	++	+	+	−	−	−	−	+	++	±	−	−	−	−	
sal6-1 SUP38-1 [η⁻]	++	+	−	−	−	−	−	+	++	++	−	−	−	−	
sal2-B SUP38-1 [η⁻]	++	+	−	−	−	−	−	+	++	++	±	±	−	+	
SUP39-1 [η⁻] or [η⁺]	++	+	++	+	±	−	−	+	++	++	−	−	−	−	
sal6-1 SUP39-1 [η⁻] or [η⁺]	++	+	±	−	−	−	−	+	++	++	−	−	−	−	
sal2-B SUP39-1 [η⁻] or [η⁺]	++	+	+	−	−	−	−	+	++	++	++	++	+	++	

All strains carry the UAG markers *met8-1*, *trp1-1*, *aro7-1*, *ilv1-1* and *ade3-26*, and the UAA marker *leu2-1*. Growth was estimated by comparing spots on plates made by inoculations with suspensions of cells. (++) good growth by 1 or 2 days; (+) good growth by 3 days; (±) some growth by 4 days; (−) no sign of growth by 6 days. The enhanced suppression levels listed in the above represent average values from large numbers of segregants with same pertinent genotype.

[a] Could not be scored past 4 days due to leaky growth.

[b] Since the *sup35-4* allele is temperature sensitive for growth, the growth of *sup35-4* containing strains was scored at 23°C.

[c] The ratio of the doubling time in −Leu media to that in the synthetic complete media was determined in representative strains as a quantitative measure of the efficiency of suppression.

ing haploid revertants by spotting on omission media where suppressor activity is required for growth. Those revertants which contained the highest suppressor activity in the haploid and the least suppressor activity in the corresponding diploid were chosen for further work since they represented the most easily scored recessive mutations. Seventeen and 22 revertants were chosen respectively from SL436-3D and L-477.

Complementation tests: The selected revertants isolated from L-477 were crossed with the selected SL436-3D revertants. Diploids were obtained and tested for suppressor activity. Loss of suppressor activity in the diploid indicated that the revertants were in different complementation groups; retention of suppressor activity in the diploid indicated that the revertants were in the same complementation group. The data from these complementation tests (Table 2) indicate that all of the 39 revertants analyzed fall into two complementation groups, called *sal2* and *sal6*.

In order to verify these complementation data, *sal2* and *sal6* mutants were intercrossed. The progeny from two crosses between independently isolated *sal2* mutants (*sal2-x asu9-1 sup45-2* × *sal2-y asu9-1 sup45-2*) all showed retention of suppressor activity. This indicates that the *sal2* mutations are allelic. Diploids from the other crosses would not sporulate. Similar attempts to determine the allelism of independently isolated *sal6* mutants failed because diploids from all the *sal6-x asu9-1 sup45-2* × *sal6-y asu9-1 sup45-2* crosses would not sporulate. The sporulation defect could be one of the pleiotrophic effects of the *sal* mutations. Thus, most diploids homozygous for the *sal* mutation would not sporulate; however, diploids heterozygous for the *sal* mutation sporulated normally. It has previously been noted that an antisuppressor mutation in *Podospora anserina* also causes the sporulation defect (COPPIN-RAYNAL 1982; DEQUARD-CHABLAT and COPPIN-RAYNAL 1984). A cross between *sal2 asu9-1 sup45-2* and *sal6 asu9-1 sup45-2*

TABLE 2

Complementation data of revertants with restored suppressor activity

Revertants from L-477	Revertants from SL436-3D																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
c	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
d	−	−															
e	−	−															
f	−	−															
g	−	−															
h	−	−															
i	−	−															
j	−	−															
k	−	−															
l	−	−															
m	−	−															
n	−	−															
o	−	−															
p	−	−															
q	+	+															−
r	+	+															−
s	+	+															−
t	+	+															−
u	+	+															−
v	+	+															−

Revertants 1–17, isolated from SL436-3D, and revertants a–v, isolated from L-477, were crossed together. All diploids are homozygous for *asu9-1* and *sup45-2*. + indicates the loss of suppressor activity in the diploid; − indicates the retention of suppressor activity in the diploid; blanks indicate that the cross was not made.

yielded some segregants with the inefficient suppressor phenotype. Since these segregants must lack both *sal* mutations, this indicates that the two allosuppressors must be at unlinked loci.

Mendelian inheritance of *sal2* and *sal6* mutations: A representative revertant from each complementation group (presumably *sal asu9-1 sup45-2*) was crossed to an *asu9-1 sup45-2* bearing strain. Segregants were tested for suppressor activity by spotting on appropriate omission media. Among the seven *sal2-B* and six *sal6-1* tetrads examined, there was a 2:2 segregation of restored suppressor activity (*sal asu9 sup45*) to the inefficient-suppressor phenotype (*asu9 sup45*). The segregants with active suppressors were sensitive to 0.5 mg/ml but resistant to 0.2 mg/ml paromomycin; in the *sal6-1* cross they were also cold sensitive at 17° (see Table 1). In contrast, segregants with inefficient suppressors (*asu9 sup45*) were sensitive to 1 mg/ml but resistant to 0.5 mg/ml paromomycin.

The *sal2* and *sal6* mutations are unlinked to *sup45* and enhance the efficiency of *sup45-2*: In order to examine the effect of the *sal* mutations on *sup45-2* in the absence of the *asu9-1* antisuppressor, representative revertants from the *sal2* and *sal6* complementation groups (presumably *sal asu9-1 sup45-2*) were crossed to a *sup45-2* bearing strain. Some segregants from these crosses exhibited unusually efficient suppressor activity when measured as described in MATERIALS AND METHODS and these were presumed to be *sal sup45-2* (see Table 1). Segregants with the inefficient-suppressor phenotype were presumed to be *asu9-1 sup45-2*. Those with normal suppressor efficiency were presumed to be *sup45-2* or *sal asu9-1 sup45-2*. These were distinguished by their different sensitivities to paromomycin and cold temperatures (see Table 1). When genotypes were assigned to segregants on the basis of this suppression efficiency and drug/cold sensitivity scheme, the *asu9-1* and *sal2-B* or *sal6-1* mutations each segregated as single independent Mendelian genes in the 12 or 14 tetrads examined respectively.

One segregant from each of these crosses which had enhanced suppressor activity (*sal2-B sup45-2* and *sal6-1 sup45-2*) was crossed with a *sup45-2* bearing strain. Enhanced suppressor activity to normal suppressor activity segregated 2:2. Furthermore, the *sal2* and *sal6* genes causing enhanced suppressor activity were unlinked to *lys2* (see Table 3). Since *sup45* and *SUP46* are linked to *lys2*, this verifies that the *sal* mutations are not alleles of these suppressors.

The same *sal sup45-2* strains used above were also crossed to a nonsuppressor bearing strain. Segregants with enhanced suppressor activity (*sal sup45-2*), normal suppressor activity (*sup45-2*), and no suppressor activity (*SUP45*$^+$) were obtained as expected, since

TABLE 3

Linkage analysis of the allosuppressors

Cross	Haploid parents	Genotype	Gene pair[a]														
			sal-lys2			*sal-hom2*			*sal-ura3*			*sal-sup*			*sal-SUP*		
			PD	NPD	T	PD	NPD	T	PD	NPD	T	PD	NPD	T	PD	NPD	T
SL-776	L-764 × L-477	*sal6-1 asu9-1 sup45-2 lys2* / + *asu9-1 sup45-2* +	1	2	4												
SL-777	L-767 × SL436-3D	*sal2-B asu9-1 sup45-2 lys2* / + *asu9-1 sup45-2* +	3	2	1												
SL-786	SL778-19B × SL428-4B	*sal6-1 sup45-2 lys2* / + *sup45-2* +	1	1	3												
SL-788	SL779-11D × SL428-4B	*sal2-B sup45-2 lys2* / + *sup45-2* +	1	2	4												
SL-787	SL778-19B × SL421-3B	*sal6-1 sup45-2* / + +										1	1	5			
SL-789	SL779-11D × SL421-3B	*sal2-B sup45-2* / + +										1	4	2			
SL-855	SL845-1B × SL853-5A	*sal6-1 asu9-1 sup45-2* + / + *ura3* / + *asu9-1 sup45-2 lys2 hom2* +	2	1	17	1	4	15	2	3	15						
SL-808	SL778-7A × SL802-2A	*sal2-B sup45-2* + / + / + / + *sup45-2 lys2 hom2 ura3*	3	1	10	1	3	10	2	1	11						
SL-836	SL-833-8A × SL421-3B	*sal6-1 sup35-4* / + +										2	1	3			
SL-840	SL834-7C × SL210-3A	*sal2-B sup35-4* / + +										2	1	3			
SL-887	SL848-2D × SL183-21C	*sal6-1 SUP38-1* / + +													1	2	4
SL-889	SL846-2C × SL421-3B	*sal2-B SUP38-1* / + +													0	2	5
SL-891	SL859-5A × SL183-21C	*sal6-1 SUP39-1* / + +													0	2	3
SL-901	SL847-2A × SL368-1B	*sal2-B SUP39-1* / + +													0	1	4
SL-924	SL902-3D × SL757-14C	*sal6-1 SUP16-o* / + +													0	0	4
SL-926	SL905-4B × SL757-14C	*sal2-B SUP16-o* / + +													3	1	3

[a] PD, NPD and T represent, respectively, parental ditype, nonparental ditype and tetratype asci.

sal2 and *sal6* are unlinked to *sup45*. The *sal2-B* and *sal6-1* mutations enhanced the growth rate of *sup45-2 leu2-1* strain about twofold on −Leu media (see Table 1). The segregants with the *sal* mutation (*sal2-B* or *sal6-1*) and without the *sal* mutation (SAL^+) in the absence of suppressor could not be distinguished from each other on the basis of suppression, cold-sensitivity, heat-sensitivity, or sensitivity to the antibiotics paromomycin, cycloheximide, trichodermin or G418. However, the genotypes of these segregants could sometimes be inferred from the genotypes of the suppressor-bearing segregants within the tetrad. Four of these inferred genotypes were verified by crossing them to a *sup45-2* strain. The putative SAL^+ strain crossed to *sup45-2* gave a 2:2 segregation of suppressors with normal efficiency to suppressorless progeny. The presumed *sal2-B* and the two presumed *sal6-1* strains crossed to *sup45-2* gave rise to some segregants with highly efficient suppressors, verifying the presence of the *sal* genes.

The *sal2* and *sal6* mutations are unlinked to *sup35* and enhance the efficiency of *sup35-4*: In order to examine the effects of the *sal* mutations on another recessive omnipotent suppressor, *sup35*, the *sal2-B* and *sal6-1* strains verified above were crossed to a *sup35-4* bearing strain. Some segregants from these crosses exhibited enhanced suppressor activity and these were presumed to be *sal sup35-4* (see Table 1). Two and one segregants, respectively, from the *sal2-B* × *sup35-4* and *sal6-1* × *sup35-4* crosses, with enhanced suppressor activity (*sal2-B sup35-4* and *sal6-1 sup35-4*) were crossed with a *sup35-4* bearing strain. Enhanced suppressor activity to normal suppressor activity segregated 2:2 in the 14 *sal2-B* and five *sal6-1* tetrads examined.

The same *sal sup35-4* strains used above were also crossed to a nonsuppressor bearing strain. Segregants with enhanced suppressor activity (*sal sup35-4*), normal suppressor activity (*sup35-4*), and no suppressor activity ($SUP35^+$) were obtained. These data (see Table 3) show that *sal2* and *sal6* are unlinked to *sup35*. Furthermore, the *sal2* and *sal6* genes, respectively causing enhanced suppressor activity in segregants from crosses SL-855 and SL-808, were unlinked to the *hom2* gene (see Table 3). Since *hom2* is linked to *sup35*, this also indicates that the *sal2* and *sal6* mutations are unlinked to *sup35*.

Effect of *sal2* and *sal6* mutations on *asu9*, *SUP38*, *SUP39* and *SUP16*: The *sal2-B* and *sal6-1* strains verified above were crossed to a strain bearing the antisuppressor mutation, *asu9-1*. The effect of the *sal* mutations on the paromomycin sensitivity and the trichodermin hypersensitivity associated with the *asu9-1* mutation was then examined in the meiotic progeny. In each of the seven tetrads examined from each cross, the drug sensitive phenotype segregated 2:2. Thus the *sal* mutations do not reverse the drug sensitivity caused by *asu9-1*.

TABLE 4

Effect of *sal2-B* and *sal6-1* on the efficiency of *SUP16-o*

Partial genotype	Complete glucose	Synthetic complete minus			
		Ade	His	Lys	Trp
SAL^+ *SUP16-o* [ψ^-]	++	−	±	±	−
sal6-1 SUP16-o [ψ^-]	++	−	+	+	−
sal2-B SUP16-o [ψ^-]	++	+	++	++	±

All strains carry the UAA markers *ade2-1*, *his5-2* and *lys1-1*, and the UAG marker *trp1-1*. Growth was estimated as described in the legend of Table 1.

The verified *sal2-B* and *sal6-1* strains were also crossed to strains bearing the recently isolated dominant omnipotent suppressors, *SUP38-1* and *SUP39-1* (J. ALL-ROBYN, E. GRIFFIN and S. LIEBMAN, unpublished results) and the serine-inserting UAA-suppressor *SUP16* (LIEBMAN, STEWART and SHERMAN 1975). Some segregants from each of these crosses exhibited enhanced suppressor activity (see Tables 1 and 4), suggesting that *sal2-B* and *sal6-1* enhance the efficiency of *SUP38-1*, *SUP39-1* and *SUP16-o*. Backcrosses of presumed *sal SUP* segregants with *SUP* bearing strains showed a 2:2 segregation of enhanced suppressor activity to normal suppressor activity. Crosses of the same *sal SUP* strains to a nonsuppressor bearing strain have confirmed that *sal2* and *sal6* were unlinked to *SUP38*, *SUP39* and *SUP16* (see Table 3).

Relationship of the *sal2* and *sal6* mutations to previously isolated *sal* mutations: Since the *sal2-B* and *sal6-1* mutations isolated in this study enhanced the efficiency of *SUP16-o* (see Table 4), we examined the relationship of *sal2-B* and *sal6-1* to the previously isolated allosuppressors *sal1*, *sal2-A*, *sal3*, *sal4* and *sal5*, that enhanced the efficiency of the UAA-suppressors (COX 1977). Diploids that were homozygous for *SUP16-o* and at least one suppressible marker and heterozygous for *sal* allosuppressors were obtained and tested for suppressor activity. The retention of suppressor activity in all diploids from the pairwise crosses (*sal2-B* × *sal1*, *sal2-B* × *sal2-A*, *sal2-B* × *sal3*, *sal2-B* × *sal4*, *sal2-B* × *sal5*, *sal6-1* × *sal1*, *sal6-1* × *sal2-A*, *sal6-1* × *sal3*, *sal6-1* × *sal4* and *sal6-1* × *sal5*) indicated that the complementation test did not work since it is impossible for *sal2-B* and *sal6-1* to be allelic to each of five different loci (*sal1-sal5*). Thus, tetrad analyses were performed. Some segregants from all the crosses, except the *sal2-B* × *sal2-A* cross exhibited weak suppressor activity characteristic of SAL^+ *SUP16-o*. In the *sal2-B* × *sal2-A* cross, all segregants exhibited enhanced suppressor activity characteristic of *sal SUP16-o* (see Tables 4 and 5). The data indicate that the *sal2-B* mutation is allelic or tightly linked to the previously isolated *sal2-A* mutation, and that the *sal6-1* mutation is unlinked to any of the *sal1-sal5* loci.

TABLE 5

The relationship of *sal2-B* and *sal6-1* to the previously isolated allosuppressors *sal1-sal5*

Cross	Haploid parents	Genotype	No. of segregants with weak suppressor activity	No. of total segregants
SL-931	SL902-3D × GF268	[ψ^-] $\frac{sal6\text{-}1}{+}\ \frac{+}{sal1}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{his5\text{-}2}{his5\text{-}2}$	8	27
SL-932	SL902-3D × GF269	[ψ^-] $\frac{sal6\text{-}1}{+}\ \frac{+}{sal2\text{-}A}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	11	28
SL-929	SL923-3C × GF266	[ψ^-] $\frac{sal6\text{-}1}{+}\ \frac{+}{sal3}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{his5\text{-}2}{his5\text{-}2}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	9	28
SL-930	SL923-3C × GF267	[ψ^-] $\frac{sal6\text{-}1}{+}\ \frac{+}{sal4}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{his5\text{-}2}{his5\text{-}2}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	4	28
SL-933	SL902-3D × GF272	[ψ^-] $\frac{sal6\text{-}1}{+}\ \frac{+}{sal5}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	4	28
SL-936	SL905-4B × GF268	[ψ^-] $\frac{sal2\text{-}B}{+}\ \frac{+}{sal1}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{his5\text{-}2}{his5\text{-}2}$	5	26
SL-941	SL928-7C × GF269	[ψ^-] $\frac{sal2\text{-}B}{sal2\text{-}A}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{ade2\text{-}1}{ade2\text{-}1}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	0	108
SL-934	SL925-3A × GF266	[ψ^-] $\frac{sal2\text{-}B}{+}\ \frac{+}{sal3}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{his5\text{-}2}{his5\text{-}2}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	9	28
SL-935	SL925-3A × GF267	[ψ^-] $\frac{sal2\text{-}B}{+}\ \frac{+}{sal4}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{his5\text{-}2}{his5\text{-}2}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	9	28
SL-938	SL905-4B × GF272	[ψ^-] $\frac{sal2\text{-}B}{+}\ \frac{+}{sal5}\ \frac{SUP16\text{-}o}{SUP16\text{-}o}\ \frac{lys1\text{-}1}{lys1\text{-}1}$	12	53

The observation that nonallelic *sal* mutations fail to complement is surprising, but not unprecedented (ATKINSON 1985). This failure to complement an effect on *SUP16-o*'s efficiency is allele-specific since *sal6-1* did not complement *sal2-A*, but did complement *sal2-B*.

Combination of the *sal2* and *sal6* mutations leads to paromomycin sensitivity and weak suppressor activity: Some segregants from a *sal2-B* × *sal6-1* cross were sensitive to 5 mg/ml paromomycin and weakly suppressed the ochre mutation *leu2-1*. These segregants were presumed to be *sal2-B-sal6-1* double mutants. To test this assumption, one such segregant was crossed with *sal2-B sup45-2*, *sal6-1 sup45-2* and *sup45-2* strains. In the crosses with the *sal2-B sup45-2* or *sal6-1 sup45-2* strains, efficient-suppressor activity (*sal2-B sup45-2*, *sal6-1 sup45-2* or *sal2-B sal6-1 sup45-2*) to no (*sal2-B SUP45*$^+$ or *sal6-1 SUP45*$^+$) or weak (*sal2-B sal6-1 SUP45*$^+$) suppressor activity segregated 2:2 among the seven tetrads examined from each cross. Normal suppressor activity was never recovered among these segregants. Furthermore, of the seven tetrads examined from the cross with *sup45-2* the following segregants were obtained: eight with no suppressor activity (*SUP45*$^+$, *sal2-B SUP45*$^+$, or *sal6-1 SUP45*$^+$); six with paromomycin-sensitivity and weak suppressor activity (*sal2-B sal6-1 SUP45*$^+$); 11 with enhanced suppressor activity (*sal2-B sup45-2*, *sal6-1 sup45-2* or *sal2-B sal6-1 sup45-2*); and three with normal suppressor activity (*sup45-2*). These results verify that the segregants which are paromomycin-sensitive and have weak suppressor activity contain both the *sal2-B* and *sal6-1* mutations.

Phenotype of the *sal2* and *sal6* mutations in the presence of the [η^+] and [ψ^+] factors: The *sal2-B* and *sal6-1* strains verified above were crossed to an [η^+] strain. The meiotic progeny were then examined for antibiotic sensitivity, temperature sensitivity, cold sensitivity, lethality and suppressor activity. There were no phenotypic differences between the *sal6-1* mutant and *SAL6*$^+$ wild-type strains among the segregants from a *sal6-1* × [η^+] cross. However, in each of the 19 tetrads examined from a *sal2-B* × [η^+] cross, the suppression of the homozygous ochre mutation *leu2-1* segregated 2:2. The suppression of *leu2-1* among all of the different segregants was weak but varied in intensity. Furthermore, when segregants with weak suppressor activity, presumed to be *sal2-B* [η^+] were cured of [η^+] by growth on 5 mM guanidine hydrochloride (LIEBMAN and ALL-ROBYN 1984), they lost suppressor activity and could not be distinguished from the *SAL2*$^+$ wild-type strains (see Table 1). This indicates that the *sal2-B* mutation leads to weak suppressor activity in the presence of the [η^+] factor.

We also examined the effect of the non-Mendelian [ψ^+] factor on *sal2-B* and *sal6-1*. The [ψ^+] factor modifies the efficiency of certain tRNA-mediated UAA-specific suppressors (COX 1971; LIEBMAN, STEWART and SHERMAN 1975; LIEBMAN and SHERMAN 1979; ONO, STEWART and SHERMAN 1979a,b) and causes lethality of some of the previously isolated *sal* mutations (COX 1977) that enhance the efficiency of UAA-specific suppressors. The verified *sal2-B* and *sal6-1* strains were crossed to a [ψ^+] strain. There were no phenotypic differences between the *sal* mutant and *SAL*$^+$ wild-type strains among the segregants from these crosses. Apparently, the *sal* mutations are not affected by the [ψ^+] factor.

Absence of centromere linkage: In order to determine whether the *sal2* and *sal6* genes are centromere linked, diploid strains were constructed to be homozygous for the omnipotent suppressor *sup45-2* and for the antisuppressor *asu9-1*. In addition, the strains were heterozygous for the centromere marker, *ura3* (5.1 cM from centromere) (MORTIMER and HAWTHORNE 1966), and for *sal2-B* or *sal6-1*. The strains were sporulated and segregants were scored for the presence of *ura3* and *sal2-B* or *sal6-1*. Among the 14 and 20 tetrads examined, 11 and 15, respectively were tetratypes (see Table 3), indicating that the *sal2* and *sal6* genes are not centromere linked since less than 67% tetratypes would indicate centromere linkage.

DISCUSSION

One successful approach for identifying genes involved in translation has been to select mutations that modify the efficiency of translational suppressors (COX 1977; LIEBMAN and CAVENAGH 1980; LIEBMAN, CAVENAGH and BENNETT 1980; ISHIGURO 1981). Such modifiers that enhance or reduce suppression efficiency are expected to alter components of the translational apparatus and may also be involved in the control of translational accuracy. Indeed, several omnipotent suppressors and modifiers have been shown to cause altered ribosomes (SMIRNOV *et al.* 1973, 1974, 1976, 1978; SURGUCHOV *et al.* 1980, 1981; LIEBMAN and CAVENAGH 1980; ISHIGURO 1981; ISHIGURO *et al.* 1981; MASUREKAR *et al.* 1981; EUSTICE *et al.* 1986).

In this paper, 39 recessive allosuppressor mutations that restore suppressor activity in *asu9-1 sup45-2* strains, have been isolated and shown to fall within two complementation groups, called *sal2* and *sal6*. In the absence of the *asu9-1* antisuppressor, the *sal2* and *sal6* genes enhance the suppressor activity of *sup45-2*. These allosuppressors also enhance the activity of other omnipotent suppressors *sup35-4*, *SUP38-1* and *SUP39-1*, and the serine-inserting UAA-suppressor *SUP16*. Although *sal* mutants were isolated as suppressor enhancers in *asu9-1 sup45-2* strains, they do not affect the activity of *asu9-1* in the absence of suppressors. The phenotypic properties of *sal*-bearing strains suggest that the allosuppressors increase translational errors caused by suppressors.

The *sal2* and *sal6* genes are not centromere linked nor are they linked to each other or any of the omnipotent suppressors *sup45*, *sup35*, *SUP38* and *SUP39*, and the UAA-suppressor *SUP16*, on which they act. The *sal2-B* mutation is allelic or tightly linked to the previously isolated *sal2-A* allosuppressor, and the *sal6-1* mutation is unlinked to any of the previously isolated allosuppressors, *sal1-sal5* (HAWTHORNE and LEUPOLD 1974; COX 1977; ONO, STEWART and SHERMAN 1979a,b) that enhance the efficiency of UAA-suppressors. Although the *sal2-B* and *sal6-1* are unlinked to the previously isolated allosuppressors *sal1*, *sal3*, *sal4* and *sal5*, they do not complement the *sal1*, *sal3*, *sal4* and *sal5*. The *sal6-1* allosuppressor complements *sal2-B*, but does not complement the previously isolated *sal2-A* allele. This unusual complementation pattern could be explained by interaction of the allosuppressor gene products and is reminiscent of that described by ATKINSON (1985). Since some allosuppressor alleles located at different loci do not complement, it is possible that more than one locus is represented by each of the complementation groups we identified. The complementation tests and tetrad analyses show that the *sal* mutations isolated in this study represent at least two unlinked loci.

The growth of omnipotent suppressor strains is inhibited by paromomycin, a drug that has been shown to cause translational misreading in yeast (SINGH, URSIC and DAVIS 1979; PALMER, WILHELM and SHERMAN 1979). Since it has been suggested that the paromomycin inhibition of omnipotent suppressor strains results from an inordinate amount of misreading (LIEBMAN and CAVENAGH 1980), we would expect *sal2* and *sal6*, which enhance these translational errors, to also enhance the suppressor-induced paromomycin sensitivity. Indeed, we have found most *sal*-omnipotent suppressor double mutants to be more sensitive to paromomycin than are omnipotent suppressors alone. The *sal2-B* and *sal6-1* mutations by themselves, without *asu9* or suppressors, do not cause sensitivity to paromomycin; however, *sal2-B-sal6-1* double mutants do cause sensitivity to paromomycin.

The non-Mendelian, [ψ^+] modifier that affects certain tRNA-mediated suppressors and some of the previously isolated *sal* mutations, does not affect the *sal2-B* and *sal6-1* mutations. However, the [η^+] modifier, that affects certain omnipotent suppressors, turns the *sal2-B* mutation into a weak suppressor.

While the mechanisms of action of *sal2* and *sal6* are unknown, it is clear that they are intimately involved in the control of translational accuracy. Even in the absence of suppressors, *sal2-B-sal6-1* double mutant strains or *sal2-B* [η^+] strains exhibit weak suppressor

activity. Perhaps the *sal* mutations fall within a structural gene coding for one of the components of the translational apparatus, such as a ribosomal protein, initiation, elongation or termination factor. Alternatively, the *sal* genes may code for products involved in the regulation or modification of the translational machinery. We are currently cloning these genes in an effort to determine the products they encode.

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Note added in proof: In the future, to avoid confusion, we will refer to our *SUP38* as *SUP44* (EUSTICE *et al.* 1986), since we recently found them to be alleles (J. ALL-ROBYN and S. LIEBMAN, unpublished results).

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