

Substitution of Serine Caused by a Recessive Lethal Suppressor in Yeast

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The *SUP-RL1* suppressor in the yeast *Saccharomyces cerevisiae* causes lethality in haploid strains but not in diploid or aneuploid strains that are heterozygous for the suppressor locus. This recessive lethal suppressor acts on amber (UAG) nutritional markers, and can cause the production of approximately 50% of the normal amount of iso-1-cytochrome *c* in disomic strains that are heterozygous for the *SUP-RL1* suppressor, and that contain the *cyc1-179* allele which has an amber codon corresponding to amino acid position 9. The suppressed iso-1-cytochrome *c* contains a residue of serine at the position that corresponds to the site of the amber codon. *SUP-RL1* was found to lie between *thr4* and *MAL2* on chromosome III, approximately 30 map units from the mating-type locus. It is suggested that the gene product of *SUP-RL1* may be a species of serine transfer RNA that normally reads the serine codon UCG, and that is represented only once in the haploid genome.

1. Introduction

Numerous nonsense suppressors in the yeast *Saccharomyces cerevisiae* have been systematically characterized with respect to their ability to suppress nutritional mutants and *cyc1* mutants which contain defined UAA (ochre) and UAG (amber) mutations. In addition, the efficiencies of suppression have been determined from the levels of iso-1-cytochrome *c* in suppressed *cyc1* mutants. These studies revealed that eight suppressors, each at a distinct locus (Gilmore, 1967), acted efficiently on UAA *cyc1* mutants, and that all of them caused the insertion of tyrosine at UAA sites (Gilmore *et al.*, 1971). Another UAA suppressor, *SUQ5-1*, was found to insert serine with a low efficiency and was found to become highly efficient with the non-Mendelian genetic factor ψ^+ which also increases the efficiencies of the UAA suppressors that insert tyrosine (Liebman *et al.*, 1975). In an extensive search for UAG suppressors that acted efficiently on *cyc1* mutants, again only eight suppressors were

uncovered and these were allelic to the eight UAA suppressors that insert tyrosine (Liebman *et al.*, 1976). Thus, so far the only efficient suppressors that have been reported are those which insert tyrosine at UAA and UAG sites and the one which inserts serine at UAA sites when the efficiency is increased by the action of the ψ^+ determinant. While it has not been excluded that other efficient UAA suppressors may be uncovered in ψ^+ strains, it does not appear as if any other efficient UAG suppressors could be normally obtained in haploid strains (Liebman *et al.*, 1976).

A search for new classes of UAG suppressors that may insert different amino acids into protein was undertaken with diploid yeast strains (Brandriss *et al.*, 1975). Brandriss *et al.* (1975) described suppressors that could not be isolated or maintained in haploid yeast but were stable in the heterozygous condition. These recessive lethals appeared analogous to the ones isolated in merodiploids of *Escherichia coli* and *Salmonella typhimurium* (Soll & Berg, 1969; Miller & Roth, 1971).

In this paper, the recessive lethal UAG suppressor, *SUP-RL1*, is shown to act efficiently on the mutant *cyc1-179* in which UAG replaces the normal AAG codon for lysine 9 in iso-1-cytochrome *c* (Stewart & Sherman, 1972), resulting in the insertion of serine at the UAG site. In addition, the position of *SUP-RL1* on the yeast genetic map has been determined more precisely.

2. Materials and Methods

(a) Mutant genes

The *cyc1-179* mutant was induced with ultraviolet light and was isolated by the chloro-lactate procedure (Sherman *et al.*, 1974). It has a UAG codon corresponding to amino acid residue position 9 (Stewart & Sherman, 1972) and is suppressible by tyrosine-inserting UAG suppressors (Sherman *et al.*, 1973; Liebman *et al.*, 1976). The symbol *CYC1+* refers to the wild-type allele for the structural gene of iso-1-cytochrome *c*.

The isolation and initial characterization of the recessive lethal UAG suppressor *SUP-RL1* has been described (Brandriss *et al.*, 1975). The symbol *sup+* refers to the wild-type allele having no suppressor activity.

The *his5-2*, *leu2-1*, *lys1-1* and *lys2-1* alleles are assumed to contain UAA mutations, since they are acted upon by UAA suppressors but not by most UAG suppressors (Gilmore, 1967; Hawthorne, 1969*a,b*; Liebman *et al.*, 1976). The *ade3-26*, *met8-1*, *trp1-1* and *tyr7-1* alleles are acted upon by UAG suppressors but not by UAA suppressors and are presumed to be UAG mutants (Hawthorne, 1969*a,b*; Sherman *et al.*, 1973; Liebman *et al.*, 1976). The *ade1*, *ade2*, *his1*, *thr4* and *can1* alleles are not suppressible by UAA or UAG suppressors. *MAL2* is a dominant gene for maltose fermentation. The symbol *mal2* refers to the inability to ferment maltose. The symbol + refers to wild-type alleles of the corresponding mutant alleles.

(b) Genetic methods

The recessive lethal UAG suppressor *SUP-RL1* could be transferred from one genetic background to another as a result of a significant delay in the onset of lethality. Spores which have inherited a recessive lethal mutation (*SUP*) germinate and proceed through several rounds of budding before growth ceases (Hawthorne & Leupold, 1974). If, during these rounds of growth, a *SUP* spore mates with a *sup+* vegetative cell or with a germinating spore which is *sup+*, the *SUP* mutation can be recovered in the diploid, since the suppressor is not lethal in the heterozygous state.

A diploid strain heterozygous for *SUP-RL1* was sporulated and the spores, still within the ascus, were mixed on a nutrient plate with an excess of cells from a haploid strain. After 2 to 3 days' incubation at 30°C, the mixture was subcloned on appropriately supplemented minimal medium to select *SUP* diploids. In cases where the *SUP* spores and the *sup+* haploids contained complementary markers, progeny diploids could be isolated by selection for prototrophy on minimal medium.

When the original diploid was heterozygous for a given marker (e.g. *cyc1-179/CYC1+*) and the desired progeny diploid was to be homozygous for that marker (e.g. *cyc1-179/cyc1-179*), the mixture of spores was allowed to germinate without addition of a haploid strain. In this case, spore-spore matings resulted and the desired diploid formed at a certain frequency. The segregation of the *SUP-RL1* gene was scored by suppression of the *trp1-1* and *tyr7-1* amber alleles. Presence of the recessive lethal suppressor was always verified by tetrad analysis, which yielded 2 live and 2 dead spores per ascus, where all the survivors were *sup+*.

Additional strains were constructed by conventional procedures of crossing, sporulation and dissection. Nutritional markers were scored by growth on synthetic glucose medium containing 0.67% Bacto-yeast nitrogen base (without amino acids), 2% glucose, 1.5% Ionagar (Wilson Diagnostics, Inc.) and appropriate amino acids. The segregation and suppression of the *cyc1-179* gene was routinely scored from the levels of cytochrome *c*, estimated by spectroscopic examinations of whole cells at low temperature (-190°C) (Sherman & Slonimski, 1964).

(c) Preparation of iso-1-cytochrome *c*

The procedure used for large-scale growth of yeast has been described by Prakash *et al.* (1974). Iso-1-cytochrome *c* was prepared by the procedure described by Sherman *et al.* (1968). Additional purification by gel filtration was according to the procedure described by Liebman *et al.* (1975).

(d) Identification of structural changes in iso-1-cytochrome *c*

The methods used for enzymic digestion, peptide mapping, sequential Edman degradation and chromatographic identification of the phenylthiohydantoin and dansyl derivatives by thin-layer chromatography, have been described (Stewart *et al.*, 1971). At the 2 degradations expected to cleave NH_2 -terminal serine from the protein, 0.25 ml 1 mM-dithiothreitol in ethylacetate was added to the extracted solution of thiazolinones, before heating in 1 N-HCl.

3. Results

(a) Suppression of the UAG mutant *cyc1-179* by *SUP-RL1*

To test whether the recessive lethal suppressor *SUP-RL1* would act on the *cyc1-179* mutant that contains a UAG (amber) codon corresponding to lysine 9 of iso-1-cytochrome *c* (Stewart & Sherman, 1972) in a way similar to the suppression observed with tyrosine-inserting as well as other UAG suppressors (Sherman *et al.*, 1973; Liebman *et al.*, 1976), it was necessary to prepare appropriate diploid and disomic strains that were heterozygous (*SUP-RL1/sup+*). The construction of some of these strains required the matings of spores with vegetative cells, and of spores with spores, as well as requiring conventional techniques of crossing, sporulation, dissection, etc. We will present the detailed procedure for construction of only the diploid strain MBD16 (*SUP-RL1/sup+ cyc1-179/cyc1-179*) from which iso-1-cytochrome *c* was extracted.

The construction of MBD16 involved two sequential matings to form, first, the heterozygote *SUP-RL1/sup+ cyc1-179/CYC1+* and then the homozygote *SUP-RL1/sup+ cyc1-179/cyc1-179*. The spores of strain DBD339 (Table 1) were mixed en masse with a haploid strain DBH422 on complete medium and mating was allowed to proceed. Prototrophic diploids that resulted from the mating of DBH422 with the spores from DBD339 were selected and purified. The genotype of one clone, MBD15, was established by tetrad analysis and is presented in Table 1. The spores of MBD15 were allowed to germinate en masse in the presence of an excess of strain DBH422,

TABLE 1
Strain numbers and genotypes

| | |
|---------|--|
| DBD339 | $\frac{a \text{ } CYC1+ \text{ } SUP\text{-}RL1 \text{ } ade1 \text{ } + \text{ } leu2\text{-}1 \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } +}{\alpha \text{ } CYC1+ \text{ } + \text{ } + \text{ } his1 \text{ } leu2\text{-}1 \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } can1}$ |
| DBH422 | $\alpha \text{ } cyc1\text{-}179 \text{ } sup+ \text{ } ade3\text{-}26 \text{ } his5\text{-}2 \text{ } lys1\text{-}1 \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } met8\text{-}1 \text{ } can1$ |
| MBD15 | $\frac{a \text{ } CYC1+ \text{ } SUP\text{-}RL1 \text{ } ade3\text{-}26 \text{ } his1 \text{ } his5\text{-}2 \text{ } leu2\text{-}1 \text{ } lys1\text{-}1 \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } met8\text{-}1 \text{ } +}{\alpha \text{ } cyc1\text{-}179 \text{ } + \text{ } + \text{ } + \text{ } + \text{ } + \text{ } + \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } + \text{ } can1}$ |
| MBD16 | $\frac{a \text{ } cyc1\text{-}179 \text{ } SUP\text{-}RL1 \text{ } ade3\text{-}26 \text{ } his1 \text{ } his5\text{-}2 \text{ } leu2\text{-}1 \text{ } lys1\text{-}1 \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } can1}{\alpha \text{ } cyc1\text{-}179 \text{ } + \text{ } + \text{ } + \text{ } his5\text{-}2 \text{ } + \text{ } + \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } can1}$ |
| MBD80† | $\frac{a \text{ } + \text{ } SUP\text{-}RL1 \text{ } mal2 \text{ } ade3\text{-}26 \text{ } his4 \text{ } leu2\text{-}1 \text{ } lys2\text{-}1 \text{ } trp1\text{-}1 \text{ } met8\text{-}1 \text{ } +}{\alpha \text{ } thr4 \text{ } + \text{ } + \text{ } + \text{ } + \text{ } + \text{ } + \text{ } trp1\text{-}1 \text{ } + \text{ } can1}$ |
| MBD126‡ | $\frac{a \text{ } thr4 \text{ } SUP\text{-}RL1 \text{ } + \text{ } ade1 \text{ } ade2 \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } can1 \text{ } leu2\text{-}1}{\alpha \text{ } + \text{ } + \text{ } mal2 \text{ } + \text{ } \text{ } trp1\text{-}1 \text{ } tyr7\text{-}1 \text{ } + \text{ } +}$ |

† Also contains either or both *ade1*/+ and *ade2*/+.

‡ Also contains either or all of *his1*/+, *his5-2*/+, and *his4*/+ and either or both *lys1*/+ and *lys2*/+.

similar to the first cross. Clones containing the suppressor were isolated and the levels of cytochrome *c* were estimated by spectroscopic examination of intact cells at low temperature. It was anticipated that the level of cytochrome *c* could be used to distinguish the following three types of expected diploids: (1) *SUP-RL1/sup+ CYC1+/CYC1+*; (2) *SUP-RL1/sup+ cyc1-179/CYC1+*; and (3) *SUP-RL1/sup+ cyc1-179/cyc1-179*. Normally without suppression, the heterozygous diploid (*cyc1-179/CYC1+*) contains approximately one-half of the normal total level, while the homozygous diploid (*cyc1-179/cyc1-179*) contains approximately 5% of the normal level, due to the presence of only iso-2-cytochrome *c*. One clone, MBD16, was found to have a level of cytochrome *c* that was intermediate to the level found in un-suppressed heterozygous (*cyc1-179/CYC1+*) and homozygous (*cyc1-179/cyc1-179*) diploids, suggesting that *SUP-RL1* was suppressing the amber mutation. The strain MBD16 was sporulated and genetic analysis confirmed the genotype *SUP-RL1/sup+ cyc1-179/cyc1-179*, which is presented in its entirety in Table 1. All the surviving spores gave rise to clones that had a cytochrome *c* level corresponding to the low amount attributed to iso-2-cytochrome *c* and therefore lacked the suppressor; spore clones containing wild-type levels of iso-1-cytochrome *c* were not observed.

In addition to construction of diploid strains that were heterozygous for *SUP-RL1* and homozygous for *cyc1-179*, appropriate crosses and dissections were carried out in order to obtain aneuploid strains that were homozygous for *cyc1-179*, that were disomic for chromosome III, and that were heterozygous for *SUP-RL1*, which is located on chromosome III. The construction of these strains involved the procedures and strains previously reported by Brandriss *et al.* (1975). When first isolated, several of the disomic strains (*cyc1-179 SUP-RL1/sup+*) contained approximately 50% of the normal level of total cytochrome *c*. However, upon extensive growth in nutrient medium, cultures appeared to give rise to mutants lacking the suppressor and to mutants with less efficient suppressors. The loss of the suppressor by mitotic segregation was also observed with the disomic strain in the first study (Brandriss *et al.*, 1975). Some mutants from cultures appeared to retain the suppressor, since the

nutritional markers *trp1-1* and *tyr7-1* remained suppressed, although the level of cytochrome *c* was only 5 to 10% of the normal amount. This appearance of strains with lowered efficiencies of suppression appears to be similar to the effect extensively studied in haploid strains, where the inhibition of growth by highly efficient UAG suppressors results in selection of strains with secondary mutations that cause partial inactivation of the suppressors (Liebman & Sherman, 1976).

Similar to the disomic strains, several diploid strains that appeared to be heterozygous for *SUP-RL1* and homozygous for *cyc1-179* also contained relatively high levels of cytochrome *c*. However, the verification of the genotypes was hampered by the lack of sporulative ability, a property that was previously noted with other strains having highly efficient UAG suppressors (Liebman & Sherman, 1976). One of the diploids MBD16 described above, contained a slightly lower level of cytochrome *c*, approximately 10 to 20% of the normal amount, and the genotype could be determined since it readily sporulated. This diploid strain was relatively stable, enabling the preparation of large quantities of cells.

(b) *Altered iso-1-cytochrome c from the cyc1-179 mutant suppressed by SUP-RL1*

Chromatographic analysis of cytochromes *c* from strain MBD16 revealed fractions corresponding approximately in position to iso-1-cytochrome *c* and iso-2-cytochrome *c*. Thus, both the increased total amount of cytochrome *c* observed in intact cells and the presence of cytochrome *c* at the elution position of iso-1-cytochrome *c* suggested that *SUP-RL1* was suppressing *cyc1-179*.

Confirmation of iso-1-cytochrome *c* in MBD16 was achieved by peptide mapping tryptic and chymotryptic digests of the chromatographically isolated protein. Peptide maps (Fig. 1) were essentially identical to those obtained previously with iso-1-cytochrome *c* that was singly substituted with serine 9 replacing normal lysine 9, in intragenic revertants of *cyc1-179* (Stewart & Sherman, 1972). Chymotryptic peptides C-2, C-2' and C-2'' were missing and were replaced by one new basic peptide. These results, for reasons discussed previously (Stewart & Sherman, 1972), indicated strongly that lysine 9 in iso-1-cytochrome *c* is replaced, and are consistent with its replacement by serine. The only other significant deviation from the normal peptide maps of iso-1-cytochrome *c* was the absence of spots attributable to the most COOH-terminal four or five amino acid residues. For unknown reasons, such an absence is frequently observed with impure iso-1-cytochromes *c* obtained in low yield; the yield of iso-1-cytochrome *c* prepared from MBD16, approximately 12 mg protein from 9 kg wet weight yeast, is only about 2% of the normal yield from normal strains. Further purification by gel filtration on Sephadex G75 failed to alter the tryptic peptide map results, and amino acid analysis of a hydrochloric acid hydrolysate of the repurified protein (unpublished results) was suggestive of slightly impure iso-1-cytochrome *c*.

The NH₂-terminal sequence of iso-1-cytochrome *c* from MBD16 was identified by sequential Edman degradation and identification of phenylthiohydantoins of amino acids released at the first ten positions and of the dansyl derivative of the residual 11th residue. The results, Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-*Ser*-Lys-Gly-, differ from the normal iso-1-cytochrome *c* sequence by the replacement of lysine 9 by serine 9, which is shown in italics. Suppression caused the translation of the amber codon as serine.

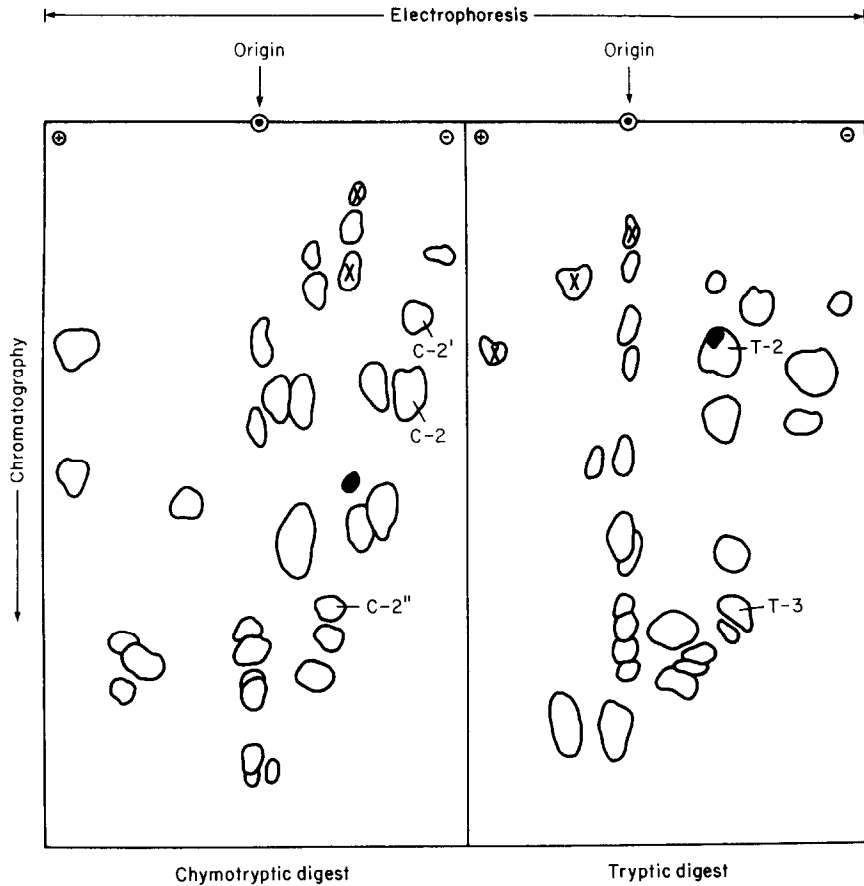


FIG. 1. Peptide maps of iso-1-cytochrome *c*. Outlined areas represent normal spots on ninhydrin-stained peptide maps of iso-1-cytochrome *c* from normal bakers' yeast. The peptide maps of the suppressed *cyc1-179* cytochrome *c* differ from the normal only as follows: normal spots T-2, T-3, C-2, C-2' and C-2'' are missing, and are replaced by new spots, centered at the filled circles; also, normal spots labeled with X, which are believed to contain the COOH-terminal 4 or 5 amino acid residues, are absent. Entirely normal results were given by staining the suppressed protein peptide maps with Ehrlich reagent for tryptophan and with Pauli reagent for histidine and tyrosine.

(c) Genetic mapping of *SUP-RL1*

On the basis of its genetic behavior in an aneuploid strain (DBA309) disomic for chromosome III, *SUP-RL1* was found to be on that chromosome (Brandriss *et al.*, 1975). In order to map its position more precisely, diploid strains were constructed to be heterozygous for *SUP-RL1* and heterozygous for the *thr4* and *MAL2* genes that have been previously mapped on chromosome III (Hawthorne & Mortimer, 1960; Mortimer & Hawthorne, 1965).

Diploid strains MBD80 and MBD126 (see Table 1) were constructed by mass matings of vegetative cells and spores in a manner similar to that described above for construction of MBD15 and MBD16. In MBD80, *SUP-RL1* was in repulsion to *thr4* and *MAL2*; in MBD126, *SUP-RL1* was coupled to *thr4* and *MAL2*.

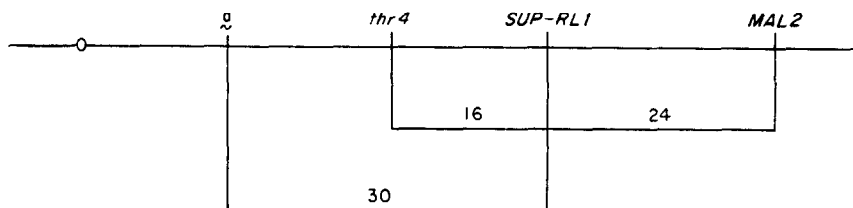
Strains MBD80 and MBD126 were sporulated and the frequencies of the genotypes

were determined from half-tetrads. The minimum number of crossovers calculated from the data clearly indicated that the gene order is *a-thr4-SUP-RL1-MAL2*.

The tetrad analysis was performed on the basis of the complete half-tetrads which result from each ascus. The distance between *SUP-RL1* and any other marker could be determined unambiguously, since all survivors were *sup+* and the ascus type was clearly either parental ditype, tetratype, or non-parental ditype. Some uncertainty did arise, however, in assigning ascus types where the other marker pairs were concerned, due to the absence of two of the four spores in each tetrad. The data for these markers, therefore, are not presented. These map distances have been published previously (Mortimer & Hawthorne, 1965). Table 2 lists the ascus types found and the distances calculated. *SUP-RL1* is approximately 16 map units from *thr4* and 24 map units from *MAL2*, and lies between these two markers.

TABLE 2
Half tetrad mapping data

| Marker pair | Number of asci | | | | | | Average map distance |
|---------------------|----------------|----|-----|----------|---|-----|----------------------|
| | MBD80:† | | | MBD126:‡ | | | |
| | PD§ | T | NPD | PD | T | NPD | |
| <i>a-SUP-RL1</i> | 12 | 23 | 0 | 6 | 4 | 0 | 30 |
| <i>thr4-SUP-RL1</i> | 24 | 11 | 0 | 7 | 3 | 0 | 16 |
| <i>MAL2-SUP-RL1</i> | 18 | 16 | 0 | 5 | 5 | 0 | 24 |



$$\dagger \text{ MBD80: } \frac{a + SUP-RL1 mal2}{a thr4 + +}$$

$$\ddagger \text{ MBD126: } \frac{a thr4 SUP-RL1 +}{a + + mal2}$$

§ PD, Parental ditype; T, tetratype; NPD, non-parental ditype.

4. Discussion

The recessive lethal amber suppressor *SUP-RL1* (Brandriss *et al.*, 1975) has been shown to cause the insertion of serine in iso-1-cytochrome *c* at the position that corresponds to the site of the UAG codon in the mutant *cyc1-179*. The suppressor can cause the production of approximately 50% of the normal amount of iso-1-cytochrome *c* in disomic strains heterozygous for the suppressor (*SUP-RL1/sup+*). This level of suppression is nearly equal to the level observed with the tyrosine-inserting UAG suppressors which can cause the production of approximately 75% of the normal level in haploid strains (Liebman & Sherman, 1976; Liebman *et al.*,

1976). The suppressor is not active on the *his5-2*, *lys1-1* or *leu2-1* alleles presumed to be UAA mutations (Gilmore, 1967; Hawthorne, 1969*a,b*; Liebman *et al.*, 1976).

Genetic analysis indicated that *SUP-RLI* is located between *thr4* and *MAL2* on chromosome III, about 30 map units from the mating-type locus. Hawthorne & Leupold (1974) have reported a recessive lethal UAG suppressor, *SUP61*, that appears to be at or near the same map position. At present it is not known whether these two suppressors are identical.

There is evidence that *SUP-RLI* determines a mutated transfer RNA. In a heterologous *in vitro* protein-synthesizing system, crude yeast supernatant made from a *SUP-RLI/sup+* disomic strain successfully translated Q β RNA containing a UAG mutation in the Q β synthetase gene (Gesteland, personal communication). Suppression was also observed with a tRNA fraction prepared from this supernatant. A high degree of suppression was observed with the *in vitro* system, where approximately equal amounts of the amber fragment and the completed synthetase were synthesized. This is consistent with our finding of efficient suppression *in vivo*.

The recessive lethality of a nonsense suppressor which is due to mutated tRNA can be explained in several ways. Suppressors which are highly efficient may act effectively on natural chain-termination codons, and may therefore interfere with the proper termination of proteins. A heterozygous diploid may be viable if the efficiency of suppression is lowered due to the decreased proportion of suppressor tRNA, and this diploid would give rise to two live and two dead spores per ascus. Over-suppression is believed to be the cause of lethality and retardation of growth when certain UAA suppressors function in the presence of the non-Mendelian factor, ψ^+ (Cox, 1971; Liebman *et al.*, 1975), or when combinations of two UAA suppressors are present in a haploid strain (Gilmore, 1967; Liebman & Sherman, 1976). Similarly, highly efficient UAG suppressors, or combinations of two UAG suppressors in a haploid strain inhibit growth (Liebman & Sherman, 1976). This explanation seems unlikely for *SUP-RLI*, since viability is observed for heterozygous strains which are either diploid or disomic, although inviability is observed for haploid strains containing *SUP-RLI*. If the degree of suppression is related to the concentration of suppressor tRNA within the cell, one would not expect to observe differences between haploid and disomic strains which are nearly equivalent in mass.

Alternatively, a recessive lethal mutation could involve an essential gene that is represented only once in the genome.

The recessive lethal UAG suppressor, *Su7*, isolated in a merodiploid of *E. coli* by Soll & Berg (1969) is an example of a mutation in an essential tRNA. The *Su7* mutation caused a single base change in the anticodon of the tryptophan tRNA, that altered the codon recognition from UGG (tryptophan) to UAG (amber) and that simultaneously caused the aminoacylation of glutamine instead of tryptophan (Soll, 1974; Yaniv *et al.*, 1974). Since *E. coli* is known to have only one gene coding for tryptophan tRNA (Hirsh, 1971), the loss of the ability to translate UGG as tryptophan is believed responsible for the recessive lethality.

The simplest interpretation of the data is that *SUP-RLI* is an altered, essential serine tRNA. There are six serine codons which in different organisms are read by a variable number of tRNAs. In yeast, ribosome binding studies have shown that the codons UCU, UCC and UCA are read by a set of tRNAs (Ser₁ and Ser₂) which have the anticodon IGA, but differ from one another in positions outside the anticodon (Zachau *et al.*, 1966; Caskey *et al.*, 1968). Another set of tRNAs (Ser_{3a} and Ser_{3b})

(Ishikura *et al.*, 1971) reads the codons AGC and AGU with the anticodon GCU. The remaining codon, UCG, which is the one related to the amber codon by one base change, may be recognized by a single tRNA having the anticodon CGA. If this species of tRNA is coded for by only a single gene in haploid yeast, its loss of function might lead to recessive lethality, since the cell could not translate UCG codons. Kruppa & Zachau (1972) have reported the presence of a small amount of a serine tRNA from yeast (Ser_x) that possibly could translate UCG. In rat liver, the anticodon CGA was determined from a partial nucleotide sequence of a minor species of serine tRNA (Rogg & Staehelin, 1971*a,b*). A single base change of G to U in the anticodon of such a tRNA that recognizes UCG would result in a tRNA that recognizes the amber codon UAG. If a similar tRNA exists in yeast, a UAG suppressor which inserts serine could arise by a G·C \rightarrow T·A transversion, the mutated anticodon being CUA, instead of the wild-type anticodon CGA.

The possibility remains, as in the case of the recessive lethal suppressor *Su7* from *E. coli* (Soll & Berg, 1969), that the mutation in *SUP-RLI* has led to mischarging of another tRNA with serine. Until the *SUP-RLI* tRNA is purified and sequenced, its identity will remain unknown.

The insertion of serine by another nonsense suppressor from yeast has recently been reported (Liebman *et al.*, 1975). This suppressor, *SUQ5-1*, originally isolated by Cox (1965), is clearly unrelated to *SUP-RLI*. The *SUQ5-1* suppressor, which maps on chromosome XVI, acts only on UAA and not on UAG codons and is efficient only in the presence of the ψ^+ genetic factor (Liebman *et al.*, 1975). It was suggested that *SUQ5-1* originated by mutation of a gene controlling a serine tRNA with a UGA anticodon; the mutated anticodon, UUA, would be enzymatically converted to SUA, which could be the anticodon of the tRNA determined by *SUQ5-1* (Liebman *et al.*, 1975).

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