Interaction of UAG Suppressors and Omnipotent Suppressors in
Saccharomyces cerevisiae

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Haploids bearing the dominant UAG suppressor, SUP7-a, and various alleles of the omnipotent suppressor sup35 were examined. The presence of the UAG suppressor reduced the efficiency of some alleles of sup35, and caused other sup35 alleles to be lethal. A nonclassical interaction of the dominant suppressor tRNA and the ribosome is proposed to explain these observations.

Recessive omnipotent suppressors in Saccharomyces cerevisiae, sup35 and sup45, act on all types of nonsense codons, UAA (ochre), UAG (amber), and UGA (opal) (4, 8, 9, 13, 17). Other suppressors are codon specific, suppressing UAA, UAG, or UGA termination 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 (1, 3, 7, 12, 14, 15). A variety of unlinked genes have been described that can alter the efficiency of omnipotent suppressors (9, 10, 18). For example, the interaction of dominant UAA suppressors and recessive omnipotent suppressors in haploids was shown to be lethal and was proposed to be due to an excessive level of suppression (18). In contrast, in a preliminary communication (S. W. Liebman and M. Cavenagh, Genetics 94:562, 1980), we reported that dominant UAG suppressors, including the tyrosine inserter (11) SUP7-a, eliminated the ability of sup35-2 to suppress the UAA marker leu2-1. This suggested that the sup35-2 allele might be a UAG mutant and the ability of sup35-2 to act as a suppressor might be suppressed by SUP7-a.

To determine whether the UAG suppressors affected a generalized reduction in the efficiency of sup35-2, it was desirable to compare the action of sup35-2 on several sup35-2-suppressible alleles in the presence and absence of UAG suppressors. Unfortunately, except for leu2-1, all of the sup35-2-suppressible markers in our strains were UAG mutants and could not be used in this test because they were suppressed by the UAG suppressors. We thus obtained a frameshift mutant, his4-713 (kindly supplied by R. Gaber and M. Culbertson [2]) and showed that it was suppressed by sup35-2. It was then possible to ask whether the tyrosyl-tRNA UAG suppressor, SUP7-a, that was previously shown to prevent sup35-2 from suppressing the UAA mutant leu2-1, would also prevent suppression of the frameshift mutant, his4-713. A diploid that was heterozygous for SUP7-a and sup35-2, and homozygous for leu2-1, his4-713, and other nonsense mutant markers, was constructed, sporulated and examined.

Table 1 shows the growth of segregants derived from a diploid, SL-798, that is heterozygous for SUP7-a and sup35-2. When this diploid was crossed with a sup35-2 + strain, the segregation of the UAG suppressor was assayed by scoring the segregants for growth on SD media lacking Trp. The complementation of the UAG suppressor was observed in the segregants grown on SD media containing Trp.

Table 2 shows the types and no. of tetrad supplemented with PD and NPD. The PD and NPD are defined to have been grown on SD media lacking Trp and containing Leu and Trp, respectively.

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Table 1. Phenotypes with respect to suppressible requirements of segregants derived from a diploid, SL-798, that is heterozygous for SUP7-a and sup35-2

<table>
<thead>
<tr>
<th>Partial genotype</th>
<th>Growth† on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-His</td>
</tr>
<tr>
<td>SUP7-a +</td>
<td>-</td>
</tr>
<tr>
<td>+ sup35-2</td>
<td>-</td>
</tr>
<tr>
<td>+ sup35-2'</td>
<td>-</td>
</tr>
<tr>
<td>SUP7-a sup35-2</td>
<td>-</td>
</tr>
</tbody>
</table>

† SL-798 is homozygous for the UAG markers ilv-1,1, met-8,1, and trp-1, for the UAA marker leu2-1, and for the frameshift marker his4-713 (see references 2 and 9 for the origin of these markers).

Table 2. Reduction in the efficiency of omnipotent suppressors caused by UAG suppressors

<table>
<thead>
<tr>
<th>Diploid†</th>
<th>Partial genotype‡</th>
<th>Types and no. of tetra‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD</td>
<td>NPD</td>
</tr>
<tr>
<td>SUP7-a +</td>
<td>SUP7-a sup35</td>
<td>SUP7-a sup35</td>
</tr>
<tr>
<td></td>
<td>+ sup35</td>
<td>+ sup35</td>
</tr>
<tr>
<td>SL-451</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>SL-798</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SL-791</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

† SL-451 and SL-791 are homozygous for the UAG markers ilv-1,1, met-8,1, and trp-1, and for the UAA marker leu2-1. See footnote ‡ to Table 1 for a description of SL-798.

‡ The sup35-4 allele is temperature sensitive for growth.

The sup35-4 allele is temperature sensitive for growth. Only ascis with four viable spores were scored. For SL-451, SL-798, and SL-791, respectively, 9 of 11, 7 of 10, and 11 of 14 ascis dissected gave four viable spores. PD (parental diplo), NPD (nonparental diplo), and T (tetra-type) ascis are marked. Since SUP7-a and sup35 are unlinked genes (8), a PD/ NPD/T ratio of 1:1:4 is expected. The excess of PD in SL-451 is probably the result of the small sample size. The genotypes of the tetrads were deduced by scoring the segregants as follows: + sup35 strains grew on -His, -Leu, -Met, and -Trp, but not on -His + Sup7-a + and Sup7-a + sup35 strains grew on -His, -Met, and -Trp, but not on -Leu media. SUP7-a sup35-4 strains were distinguished from SUP7-a + strains on the basis of their temperature sensitivity (amino acid abbreviations are defined in footnote ‡ to Table 1). The distinction between SUP7-a sup35-2 and SUP7-a + strains was inferred from the genotypes of the other segregants in the tetrad.
dissected, and segregants were examined for suppressor activity. Growth was estimated by comparing spots on agar-medium made by inoculations with suspensions of cells on synthetic glucose medium containing 0.67% (wt/vol) yeast nitrogen base (without amino acids), 2% (wt/vol) Bacto-Agar (both from Difco Laboratories), and appropriate amino acids (16). When SUP7-a was coupled to sup35-2, leu2-1 and his4-713 were never suppressed. In the absence of SUP7-a, sup35-2 suppressed leu2-1 and his4-713 efficiently (Tables 1 and 2). This result verified that SUP7-a reduced the efficiency of sup35-2.

The lethal effects observed with SUP7-a are reminiscent of those described by Tev-Avanesyan et al. (18), when UAA suppressors were combined with omnipotent suppressors. Indeed, we also found that when the UAA suppressor SUP7-a (5) was crossed to sup35-2, all SUP7-a sup35-2 segregants were inviable (Table 3).

We have shown that various codon-specific suppressors can alter the efficiency and viability of several alleles of the omnipotent suppressors sup35 and sup45. These results suggest that the codon-specific suppressors interact with the omnipotent suppressors not by the classical suppression mechanism, but rather by some other interaction of the suppressor tRNA and the ribosome.

The fact that codon-specific suppressors can reduce the efficiency of certain alleles of the omnipotent suppressors and can cause death in other cases calls into question the previous hypothesis (18) that the lethality is due to excessive suppression. Why the same UAG suppressor acting on different sup35 alleles can sometimes cause reduced suppression, and other times cause lethality, is unknown. Likewise, the observation that the UAG and UAA alleles of the SUP7 suppressor, which differ by only a single base (6, 15), exert different effects on sup35-2 is unexplained.

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LITERATURE CITED

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