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# Substitution of an invariant nucleotide at the base of the highly conserved '530-loop' of 15S rRNA causes suppression of yeast mitochondrial ochre mutations

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## ABSTRACT

We have determined the nucleotide sequence alteration in the 15S rRNA gene of a *Saccharomyces cerevisiae* strain carrying the previously described mitochondrial ochre suppressor, *MSU1*. The suppressor contains an A residue at position 633 of the yeast mitochondrial sequence, in place of the wild-type G. This position, located in the highly conserved region forming the stem of the '530-loop', corresponds to G517 of the *Escherichia coli* 16S rRNA and is occupied by G in all other known small rRNA sequences. This finding strongly supports the previous conclusions of others that the 530-loop region plays an important role in enhancing translational accuracy.

## INTRODUCTION

The phenomenon of informational suppression is due to mutations that affect components of the gene expression machinery required for accurate translation. These suppressor mutations cause errors during translation that can compensate for specific kinds of mutational errors in particular genes. Suppressor mutations have proven very useful in understanding the function of tRNAs (1) and have also identified ribosomal proteins that play a role in translational acccuracy (2, 3, 4, 5, 6). More recently, a UGA-specific suppressor mutation in the 16S rRNA of *Escherichia coli* has been isolated and characterized at the sequence level, directly demonstrating a role for this rRNA as well in promoting accuracy (7).

We have previously described a mutation in the yeast (*Saccharomyces cerevisiae*) mitochondrial genetic system that causes suppression of ochre (UAA) mutations (8). Interestingly, this mutation, termed *MSU1*, mapped to the region of mtDNA encoding the mitochondrial 15S rRNA (8). Here we report the identification of the *MSU1* mutation by DNA sequence analysis as a base substitution in a position that has been found to be invariant in all wild-type rRNAs examined to date.

## MATERIALS AND METHODS

*Yeast strains.*

The wild-type 15S rRNA gene analyzed in this study was obtained from the strain V25T (8). V25T contains the mtDNA of the *oxi1* mutant strain AB1-4D/V25 (9), which was shown to contain an ochre codon (8). The ochre suppressor form of the 15S rRNA gene, *MSU1* , was obtained from the strain V25T-R5, which was isolated as a spontaneous respiring revertant of strain V25T (8).

*Isolation and sequence analysis of the 15S rRNA genes.*

Purified mtDNA was isolated from each strain as previously described (10), digested with the restriction enzyme MspI and fractionated by agarose gel electrophoresis. The 2kb fragment containing the entire15S rRNA gene (11) and 347 bp of flanking DNA was isolated

 

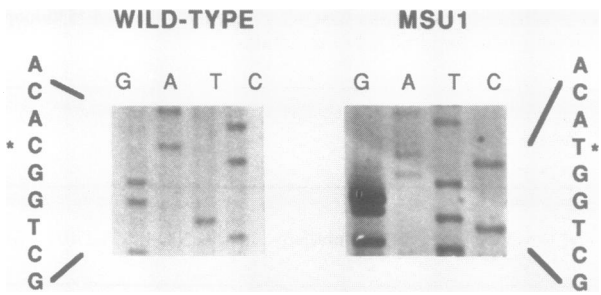


**Figure 1:** Identification of the 15S rRNA base substitution corresponding to the ochre suppressor *MSU1*. Portions of the DNA sequence autoradiograms showing the sequence complementary to the 15S rRNA are presented. The mutation affected the position indicated by the asterisks.

from each strain and ligated into the AccI site of the plasmid Bluescript, obtained from Stratagene Inc. Nested deletions were generated in both directions in each plasmid (12), and used as templates for DNA sequencing reactions (13, 14). The entire sequence of each fragment (1,998 bp) was determined by analysis of both strands.

## RESULTS

To identify the ochre suppressing mutation, *MSU1*, we determined and compared the complete DNA sequences of the mitochondrial 15S rRNA genes from a strain carrying the non-suppressing (wild-type) gene and an ochre-suppressing strain derived from the isogenic wild-type by spontaneous mutation (8). The only difference between these sequences was a substitution of the wild-type G residue at position 633 (using the numbering system for yeast mitochondria of ref. 11) by an A residue in the suppressor mutant. This substitution was observed in the DNA sequence of both DNA strands and is shown for the strand complementary to the 15S rRNA in Fig. 1. This substitution must represent the *MSU1* mutation, and not a simple polymorphism, since the two genes are directly related to each other by spontaneous mutation, and since this was the only difference observed in the DNA sequence of the region in which *MSU1* was mapped genetically (8).

Polymorphisms between the 15S rRNA genes of different yeast strains have been previously noted (11). Our wild-type sequence for the strain V25T is identical to that reported for the strain 777-3A (11), except that the bases 1136−1145 are 5′-AGATTAAGTT-3′ (V25T) instead of 5′-AGATAATGTT-3′ (777-3A). A third polymorphic sequence in this region is present in two other yeast strains (15, 16). However, none of these naturally occuring polymorphisms are associated with ochre suppression.

## DISCUSSION

Sequences of rRNA from all species examined exhibit a large number of highly conserved regions that reflect important functional domains (17). The *MSU1* ochre suppressor mutation occurs in one such highly conserved region, usually drawn as the stem of the '530-loop' of small rRNAs (Fig. 2). The position of this G to A mutation in the yeast mitochondrial sequence corresponds to G517 of the *E. coli* 16S rRNA. Interestingly, a recent comparison of 106 known sequences revealed that this G residue is invariant in procaryotic, eucaryotic and organellar small rRNAs (18). [The only reported exception was in the discontinuous

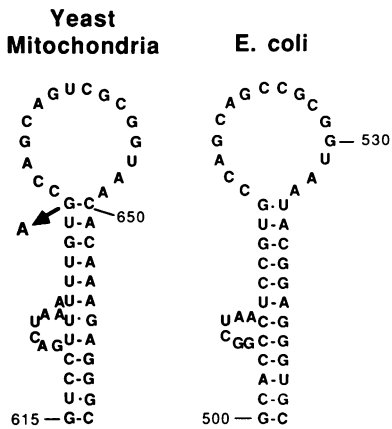


**Figure 2:** Comparison between the homologous '530-loop' regions of yeast mitochondrial and *E. coli* small rRNAs. The G to A substitution in the ochre suppressor *MSU1* is indicated by the arrow. The numbering of the mitochondrial sequence follows reference 11.

mitochondrial small rRNA of the ciliate *Tetrahymena pyriformis* strain ST, in a sequence derived by comparison of the ciliate mtDNA sequence with the *E. coli* rRNA sequence (19). However, a more reliable direct sequence determination of both the mitochondrial rRNA and mtDNA from *T. pyriformis* strain ST had previously revealed a G at the position corresponding to the site of the *MSU1* mutation (20).] Although ribosomes with the *MSU1* mutation must carry out translation reasonably well, as judged by the respiratory growth of yeast strains containing them (8, unpublished results), the evolutionary invariance of this base argues that it has an important function.

Several experimental approaches have already strongly indicated that the 530-loop region plays a functionally important role in translation. Three bases in this loop (G529, G530, U531 of *E. coli* ) are protected from chemical attack by codon-anticodon interaction on the ribosome, and a fourth (A532) is protected by the binding of tRNA alone (21). Binding of the error-inducing drugs neomycin, paromomycin, gentamycin or kanamycin to ribosomes enhances the chemical reactivity of base C525 (22). A base substitution in the 530-loop (A523 to C), originally identified as causing streptomycin resistance in *Chlamydomonas* chloroplasts (23), was generated in *E. coli* and conferred resistance to the error-inducing effects of streptomycin as well as neomycin, gentamycin and kanamycin (24). Since these effects involve codon-anticodon interactions and error-inducing antibiotics it is highly likely that they reflect functions that enhance translational accuracy. In this connection it is clearly also of interest that the ribosomal proofreading protein S4 of *E. coli* (4) interacts with the 530-loop (27). The 530-loop region appears to be located on the opposite side of the 30S particle from the region where codon-anticodon interactions take place (25, 26, 21), and does not correspond to the primary binding sites of the error-inducing antibiotics (22). Thus, the above observations are thought to be reflections of allosteric interactions between the 530-loop region and the decoding region that enhance translational accuracy (21, 22, 24).

Our data on the nature of the *MSU1* suppressor mutation provide direct support for the hypothesis that the 530-loop region plays an important role in regulating translational

accuracy *in vivo* , although they do not point to a particular mechanism for suppression. One possibility is that the mutation could generally reduce discrimination at the A site between correct and incorrect tRNAs. A second possibility is that the mutation could interfere with the action of a release factor, thereby promoting readthrough of stop codons. If the first possibility were true, one might expect *MSU1* to suppress a wide spectrum of different kinds of mutations. However, in a limited test of allele specificity, *MSU1* failed to suppress four frameshift mutations and two missense mutations in the *oxi1* gene (8). We have also tested *MSU1* against the *cob* amber mutation M611 (28), but it similarly failed to cause detectable suppression. While these data tend to suggest that *MSU1* may be specific for ochre mutations, it must be emphasized that a rigorous test of allele specificity (against ochre, amber, missense and frameshift mutations affecting the same codon) cannot presently be carried out in yeast mitochondria. The only other defined rRNA suppressor mutation, *rrsB* (suUGA-ΔC1054) of *E. coli* , is highly specific for UGA mutations (7). Its mechanism of action is unknown.

In addition to *MSU1*, two other informational suppressors in yeast mitochondria have been mapped genetically to the region of mtDNA encoding the 15S rRNA. One of them, isolated as an ochre suppressor, has been reported to suppress at least one frameshift mutation as well (29). The other suppressor was identified as a naturally occuring genetic determinant in one wild-type strain (30). This suppressor appears to act specifically on frameshifts, and is partially responsible for the 'leakiness' of many yeast mitochondrial frameshift mutations (28, 31).

Genetic selections applied to organellar systems have proven to be very effective in generating mutations in rRNAs. In addition to suppressor mutations, many drug resistant mutants and conditional-function mutants have been isolated and analyzed (15, 23, 32, 33, 34, 35, 36, 37, 38, 39). In several cases, base substitutions identified in organellar mutants have had similar phenotypic effects when generated in *E. coli* (24, 40), demonstrating that organellar systems can provide a useful guide to understanding translation systems in general. Thus, it is likely that a better understanding of the mechanism of *MSU1* ochre suppression, obtained by making the homologous mutation in *E. coli* and studying its effects *in vivo* and *in vitro*, will shed light on rRNA functions that promote accurate translation.

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