Identification of a tRNA^{Gln} ochre suppressor in *Saccharomyces cerevisiae*

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Anticodon mutations that result in ochre (UAA) codon suppression have been observed for tRNA^{Leu}, tRNA^{Tyr}, and tRNA^{Ser} loci of *S.cerevisiae* (1). Extensive efforts have failed to identify ochre-suppressing mutations in genes encoding other tRNA species, although over-expression of a wild-type tRNA^{Gln} results in some ochre-suppression activity (2). Here we report the identification of a structural gene encoding a tRNA^{Gln}, with an anticodon mutation that results in ochre codon suppression.

Previously we identified a dominant mutation that suppresses the mating defect associated with an ochre mutation of the gene encoding the yeast a-factor receptor, *STE3* (3). Interestingly, this suppressor mutation, *SRM2-2*, was associated with increased sensitivity to a-factor. To investigate the role of the *SRM2* gene we sought to clone the dominant *SRM2-2* allele. A genomic library was constructed from the yeast strain SY1001 (*MATα ste3-1,SRM2-2*), in a centromere based shuttle vector containing the *URA3* gene, pRS316 (4). The plasmids prepared from the library were used to transform the yeast strain VQ3U (*MATα ste3-1,ura3*) and uracil prototrophs were selected. Transformants were subsequently screened for plasmid dependent mating competence. Ten plasmids, containing distinct but overlapping inserts, were identified in this manner. Like the original *SRM2-2* mutant strain, transformants carrying these plasmids were associated with an increased sensitivity to a-factor. These plasmids led to allele-specific suppression: none could suppress the mating defect associated with *ste3-28* (missense), *ste3-115* (frameshift), or *ste3Δ* (deletion). Although preliminary tetrad analysis had suggested that *SRM2-2* could suppress the missense and frameshift alleles (3), we have carried out a more complete analysis and find that *SRM2-2* can only suppress the *ste3-1* ochre allele (data not shown). Thus, the defining *SRM2-2* mutation and the isolated plasmids exhibit the same features: they suppress only the *ste3-1* mutation and they confer increased sensitivity to a-factor.

Restriction mapping and subcloning of the genomic insert of a representative plasmid, indicated that a small fragment of approximately 250 bp allowed suppression of *ste3-1*. Sequence analysis of this fragment demonstrated that it contained a region that showed significant sequence identity to an *S.cerevisiae* tRNA^{Gln} (5), but encoded for an anticodon capable of ochre codon recognition. However, the sequence of the *SRM2* locus is clearly distinct because the regions flanking tRNA^{Gln} genes differ markedly (Figure 1).

To verify that the anticodon mutation was responsible for the suppression phenotype, we isolated the wild-type *SRM2* gene by gap repair transformation (6). The isolated *SRM2*⁺ plasmid was

not able to suppress the *ste3-1* mutation. The *SRM2-2* allele was also able to suppress another ochre mutation, *ade2-101*, but only weakly and only when on a multicopy 2 μ based vector, YEp352 (7). No suppression of *ade2-101* was observed when *SRM2-2* was carried on a centromere based vector, or when the wild-type *SRM2* gene was present on a 2 μ based vector. Thus, the ochre suppression conferred by *SRM2-2* can be relatively weak, which may explain why previous studies failed to isolate ochre-suppressing alleles of tRNA^{Gln} loci. We suggest that suppression of the mating defect of *MATα ste3-1* mutants may provide a sensitive method for the identification of other novel ochre-suppressor tRNA mutations.

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Figure 1. Reverse strand encoding the *SRM2-2* gene and an alignment with a sequence from a published tRNA^{Gln} gene (see text, ref. 5). The nucleotides encoding the predicted anticodon loop have been underlined.

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