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A novel type of +1 frameshift suppressor: a base substitution in the anticodon stem of a yeast mitochondrial serine-tRNA causes frameshift suppression

Alexander Hüttenhofer[1,2], Brigitte Weiss-Brummer[2], Guy Dirheimer[1] and Robert P.Martin[1]

[1]Institut de Biologie Moléculaire et Cellulaire du CNRS, Laboratoire de Biochimie, and Université Louis Pasteur, 15 rue R. Descartes, 67084 Strasbourg, France and [2]Institut für Genetik und Mikrobiologie, Universität München, Maria-Ward-Strasse 1a, D-8000 München 19, FRG

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We have identified a spontaneous mitochondrial mutation, *mfs-1* (mitochondrial frameshift suppressor-1), which suppresses a +1 frameshift mutation localized in the yeast mitochondrial *oxi1* gene. The suppressor strain exhibits a single base change (C to U) at position 42 of the mitochondrial serine-tRNA (UCN). To our knowledge, this is the first reported case showing that a mutation in the anticodon stem of a tRNA can cause frameshift suppression. The expression and aminoacylation of the mutant $tRNA^{Ser}_{(UCN)}$ are not significantly affected. However, the base change at position 42 has two effects: first, residue U27 of the mutant tRNA is not modified to pseudouridine as observed in wild-type $tRNA^{Ser}_{(UCN)}$. Second, the base change and/or the lack of modification of U27 leads to an alteration in the secondary/tertiary structure of the mutant tRNA. It is possible that there are such structural changes in the anticodon loop that enable the tRNA to read a four base codon, UCCA, thus restoring the wild-type reading frame.

Key words: +1 frameshift suppressor/anticodon stem alteration/$tRNA^{Ser}_{UCN}$/yeast mitochondria

Introduction

The roles of translational macromolecules in maintaining the accuracy of the reading frame can be studied by external suppressors of frameshift mutations. The accuracy of reading frame maintenance is mostly affected by mutations in tRNAs (for a review, see Roth, 1981) but there are also some instances in which mutant forms of elongation factors-Tu (EF-Tu) in *Escherichia coli* and *Salmonella typhimurium* cause suppression of frameshift mutations (Hughes *et al.*, 1987).

Recently, we have isolated the suppressor mutation *mfs-1* (mitochondrial frameshift suppressor-1), which is capable of suppressing the mitochondrial frameshift mutation M5631 in *Saccharomyces cerevisiae* strain HS2-5631 (Weiss-Brummer *et al.*, 1989). The M5631 mutation has been shown previously to be a +1T insertion in the mitochondrial gene *oxi1*, which codes for subunit II of cytochrome *c* oxidase (Fox and Weiss-Brummer, 1980).

The suppressor mutation *mfs-1* was isolated in a strain, HS2-5631, carrying the par^r-*454* mutation to quieten a background of natural frameshifting (~20%) in the original mutant M5631 (Weiss-Brummer *et al.*, 1989). The par^r-*454* mutation within the mitochondrial 15S rRNA gene was shown to reduce the level of natural frameshifting from 20 to ~10%, thereby facilitating isolation of suppressors (Weiss-Brummer and Hüttenhofer, 1989). As the presence of the par^r-*454* mutation would make it difficult to evaluate suppression under conditions of normal ribosome function, we have also tested the suppressor ability of *mfs-1* in a par^s (wild-type allele of par^r*454*) background. It could be shown that the *mfs-1* suppressor acts in both par^s and par^r-*454* backgrounds with equal efficiency and is allele specific for the M5631 mutation. No other known mitochondrial frameshift, missense or nonsense mutations are suppressed by *mfs-1* (Weiss-Brummer *et al.*, 1989).

The suppressor mutation *mfs-1* is mitochondrially inherited (Weiss-Brummer *et al.*, 1989). While nuclear genes code for most of the components of the mitochondrial protein synthesizing system, the mitochondrial DNA codes for two rRNAs (21S RNA and 15S RNA), 24 tRNAs, a 9S RNA involved in tRNA processing, ribosomal protein (var1) and some polypeptide components of the respiratory chain (for a review, see Dujon, 1983). The mutation *mfs-1* was mapped by genetic and molecular analyses to the $tRNA^{Ser}$-var1 region of the mitochondrial genome (Weiss-Brummer *et al.*, 1989).

This paper shows that an alteration in the mitochondrial $tRNA^{Ser}_{(UCN)}$ causes suppression of the mitochondrial +1 frameshift mutation M5631. Many suppressors of +1 frameshift mutations have been identified as tRNAs with increased anticodon loop size (Bossi and Smith, 1984; Curran and Yarus, 1987). The data presented here show that frameshift suppression can also be caused by a tRNA with normal anticodon loop size, but with an alteration in the anticodon stem.

Results

Sequence analysis of the mfs-1 suppressor mutation

The *mfs-1* suppressor mutation has been mapped to a 2 kb fragment containing the $tRNA^{Ser}$-var1 region (Weiss-Brummer *et al.*, 1989). This region codes for the UCN reading $tRNA^{Ser}$ and for the var1 protein, the only mitochondrially encoded ribosomal protein. The 2 kb *Hae*III fragments of the suppressor strain SEG15 (carrying the *mfs-1* mutation) and of the original strain HS2-5631 (for genotypes, see Table I) were cloned into M13mp8 and sequenced over their entire lengths (see Materials and methods). Comparison of the nucleotide sequences obtained from each strain revealed only one difference: a base alteration from C to T at position 124 of the region coding for the $tRNA^{Ser}_{(UCN)}$ in the suppressor strain SEG15 (Figure 1; Figure 2). The C to T base alteration of the $tRNA^{Ser}_{(UCN)}$ gene is located

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Table I. Genetic markers and origin of yeast strains

Strain	Genotype		Remarks	Reference
	Nuclear	Mitochondrial		
HS2-5631	a*his1, trp2*	*rho*$^+$, *mit*$^-$ *cap*^{r}*-321, par*^{r}*-454*		Weiss-Brummer *et al.* (1987)
SEG15	a*his1, trp2*	*rho*$^+$, *mit*$^-$ cap^r*-321, par*^{r}*-454*	derived from HS2-5631	Weiss-Brummer *et al.* (1989)

Origin of markers: *cap*^{r}*-321*, Coen *et al.* (1970); *par*^{r}*-454*, Kutzleb *et al.* (1973).

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   1 CCGGAACCCCGAAAGGAGTTTATTTAATATTTATATTTATATTAATATTTATATTTATATTTATATTTATATTCC
                                                  T
  76 TCTTAAGGATGGTTGACTGAGTGGTTTAAAGTGTGATATTTGAGCTATCATTAGTCTTTATTGGCTACGTAGGTT
 151 CAAATCCTACATCATCCGTAATAATACATATATATAATAATAATTTTAATATTATTCCTATAAAAATAAAATAAA
 226 TAAATAAATAATAATAATTAATTAATTAATTAATTTTAATAAATATAAAATATATAAAAATAATAATAATAATAA
 301 TTATTATTTTAATAATATTATTTATATAATAGTCCGG
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Fig. 1. Nucleotide sequence of the tRNA$^{Ser}_{(UCN)}$ gene region of original strain HS2-5631. The sequence of the var1 gene region [downstream from the tRNA$^{Ser}_{(UCN)}$ gene] is not shown in the figure because it is identical to previously published sequences (Hudspeth *et al.*, 1982; Zassenhaus *et al.*, 1983). The tRNA gene sequence is underlined. The base substitution from C to T in the tRNA^{Ser} gene of suppressor strain SEG15 is boxed. The mutation is localized at position 124 of the shown sequence. The mutational site corresponds to position 42 in the generalized tRNA cloverleaf structure.

within the anticodon stem of the tRNA (position 42 in the numbering of the generalized cloverleaf structure; Sprinzl *et al.*, 1985) and leads to the alteration of a G−C base pair to the thermodynamically weaker G−U pair within this stem (Figure 3).

Frameshift suppression site in suppressor strain SEG15

The *mfs-1* suppressor was shown to suppress specifically the M5631 frameshift mutation in the mitochondrial *oxi1* gene of the yeast *S.cerevisiae* (Weiss-Brummer *et al.*, 1989). To determine whether a mutated tRNA$^{Ser}_{(UCN)}$ could act as a frameshift suppressor of the M5631 mutation, the altered *oxi1* reading frame was analysed with respect to the presence of UCN codons. As can be seen in Figure 4, the first codon after the mutational site of the mutant M5631 *oxi1* reading frame (which differs from the wild-type reading frame by the insertion of a T base) is a codon UCC, which is decoded by the tRNA$^{Ser}_{(UCN)}$.

If the frameshift suppression in strain SEG15 is purely the result of the base exchange within the tRNA$^{Ser}_{(UCN)}$ gene, either the expression or the biological function of this tRNA should be affected. To test this, we analysed the transcription, processing, structure and aminoacylation of mutant and wild-type tRNA$^{Ser}_{(UCN)}$.

Analysis of tRNA$^{Ser}_{(UCN)}$ transcripts

To determine whether the base alteration from C to U at position 42 within the anticodon stem has an effect on transcription, processing or stability of the tRNA, we analysed the tRNA$^{Ser}_{(UCN)}$ transcripts in the original strain HS2-5631 and in suppressor strain SEG15. Northern analysis showed that both strains contain the tRNA$^{Ser}_{(UCN)}$ transcript at about the same level and with the same size as determined by separation on a 10% polyacrylamide−8 M urea gel (data not shown). S1 nuclease mapping revealed that both 5′ and 3′ termini of the suppressor tRNA are the same as in the mature tRNA of the original strain HS2-5631 (data not shown). These results indicate that the mutation within the anticodon stem of the tRNA affects neither transcription of the gene nor processing of the transcript.

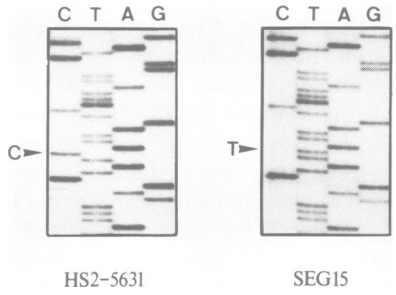

Fig. 2. Sequencing gel demonstrating the point mutation in the tRNA$^{Ser}_{(UCN)}$ gene. The autoradiogram represents part of the non-coding strand of the tRNA$^{Ser}_{(UCN)}$ gene in strain HS2-5631 (left panel) and SEG15 (right panel). The products from four enzymatic sequencing reactions (C, T, A, G) are indicated at the top of each lane. The mutational site (C in HS2-5631 and T in SEG15) is indicated by an arrow.

Two-dimensional PAGE of tRNAs from suppressor and original strain

It has been shown by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and subsequent sequencing of the respective tRNA spots that two UCN-reading tRNA^{Ser} species are present in wild-type strains of *S.cerevisiae* (Martin *et al.*, 1982). These species—tRNA$^{Ser}_2$ and tRNA$^{Ser}_3$—are transcribed from a single gene in the var1 region corresponding to the mutated tRNA$^{Ser}_{(UCN)}$ gene in suppressor strain SEG15. The two isoacceptors differ by a single nucleoside modification at position 27: tRNA$^{Ser}_2$ contains a modified uridine (i.e. pseudouridine) while tRNA$^{Ser}_3$ lacks this modification at position 27 (Martin *et al.*, 1982; Figure 3).

2D-PAGE analysis of the tRNAs showed that the two spots of wild-type tRNA$^{Ser}_2$ and tRNAS$^{Ser}_3$ in strain HS2-5631 (Figure 5A) are absent in the suppressor strain SEG15 and

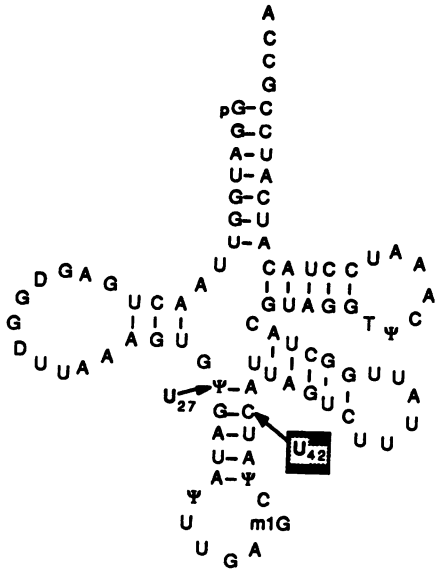

Fig. 3. Cloverleaf structure of the $tRNA^{Ser}_{(UCN)}$ deduced from its gene sequence and base modifications according to Martin *et al.* (1982). Two tRNA species—$tRNA^{Ser}_2$ and $tRNA^{Ser}_3$—which are transcribed from a single gene—are observed in wild-type cells. $tRNA^{Ser}_2$ differs from $tRNA^{Ser}_3$ in that it contains pseudouridine (Ψ) at position 27 instead of uridine (Martin *et al.*, 1982). This modification site and the base exchange (C → U at position 42) in suppressor strain SEG15 are indicated by an arrow.

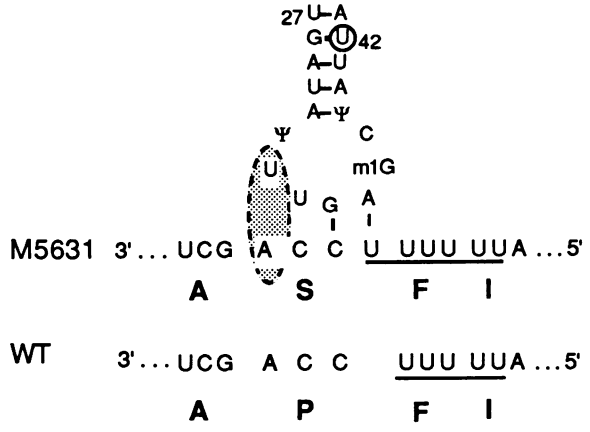

Fig. 4. Section of the *oxi1* mRNA sequence (in 3′−5′ orientation) of wild-type (WT) and mutant M5631, according to Fox and Weiss-Brummer (1980); the section shows the site of the M5631 mutation, a +1U insertion in a consecutive series of 5U residues (underlined); below the respective mRNA sequences the deduced amino acid sequences are indicated. A model of suppression of the frameshift mutation M5631 by the *mfs-1* $tRNA^{Ser}$ is shown above (see Discussion).

are replaced by a single new spot in close vicinity of the $tRNA^{Leu}$ spot (2′ in Figure 5B).

RNA sequence analysis of suppressor and wild-type $tRNA^{Ser}_{(UCN)}$

To confirm that the new spot (2′ in Figure 5B) in the suppressor strain does indeed represent the mutated $tRNA^{Ser}_{(UCN)}$, we performed limited digestion of this tRNA and of wild-type $tRNA^{Ser}_2$, $tRNA^{Ser}_3$, and $tRNA^{Leu}$ with ribonuclease T1. As shown in Figure 6A, the T1 pattern of the new tRNA spot in suppressor strain SEG15 is identical to the T1 pattern of wild-type $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$, which demonstrates that this spot represents the mutated $tRNA^{Ser}$. Thus, while in the original strain HS2-5631, two isoacceptors are present—$tRNA^{Ser}_2$ and $tRNA^{Ser}_3$—the suppressor strain contains only a single $tRNA^{Ser}_{(UCN)}$ species, with an altered mobility compared to the respective wild-type tRNAs. This implies that the C to U mutation seems to have an influence on the conformation of the suppressor tRNA, here referred to as *mfs-1* $tRNA^{Ser}$.

As wild-type $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$ of the original strain HS2-5631 can be separated on a 2D gel due to the modification or non-modification of the uridine at position 27, we analysed the *mfs-1* $tRNA^{Ser}$ with respect to this modification site. Since the nucleoside which is modified at position 27 is a uridine, only the U reaction of the chemical sequencing was performed. According to Peattie (1987), the absence of an expected band in the lane of a chemically generated sequence pattern indicates that a modified base is present. In the sequence pattern of the U-sequencing reaction of the *mfs-1* $tRNA^{Ser}$ the band at position 27 is present, indicating that this residue is not modified (Figure 6B). Control experiment with $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$ from the original strain HS2-5631 were performed as described above, confirming the modification of $tRNA^{Ser}_2$ at position 27 and the lack of modification of $tRNA^{Ser}_3$ at this position (Figure 6B; Martin *et al.*, 1982).

These data show that in suppressor strain SEG15 only one UCN reading $tRNA^{Ser}$ species (the *mfs-1* $tRNA^{Ser}$) is present, compared to two in the wild type. The *mfs-1* $tRNA^{Ser}$ harbours a C to U base transition at position 42 within the anticodon stem; in addition, this alteration prevents the uridine at position 27 of the suppressor tRNA from being modified to pseudouridine, as occurs in the wild-type strain (see Figure 3).

Analysis of mfs-1 $tRNA^{Ser}$ on a non-denaturing gel

To determine the structural differences between wild-type and suppressor $tRNA^{Ser}_{(UCN)}$, total mitochondrial RNA from strains HS2-5631 and SEG15 was separated on a 10% polyacrylamide gel without urea. In this gel electrophoresis system, separation of tRNAs is expected to occur mainly on the basis of their conformational stability. After separation, tRNAs were transferred to a nylon membrane (Zeta Probe, Bio-Rad) and hybridized with a nick-translated DNA probe containing the $tRNA^{Ser}_{(UCN)}$ gene (see Materials and methods). As can be seen in Figure 7A, two tRNA bands are present in the original strain HS2-5631, representing $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$ respectively (Martin *et al.*, 1982). In suppressor strain SEG15, two closely migrating bands are visible (Figure 7B, 2a′ and 2b′). Nevertheless, as shown by 2D gel electrophoresis and subsequent sequence analysis (Figures 5 and 6), only one $tRNA^{Ser}_{(UCN)}$ species is present in strain SEG15, the *mfs-1* $tRNA^{Ser}$. The existence of two *mfs-1* $tRNA^{Ser}$ bands on a non-denaturing gel could be explained by differential nucleoside modification of the tRNA in a way not detectable by sequencing, e.g. because the alterations were covered by the contaminant $tRNA^{Leu}$ (see Figure 6). Alternatively, the two closely migrating tRNA bands (Figure 7B) could reflect two different conformations of the *mfs-1* $tRNA^{Ser}$, which might have different decoding abilities (see Discussion).

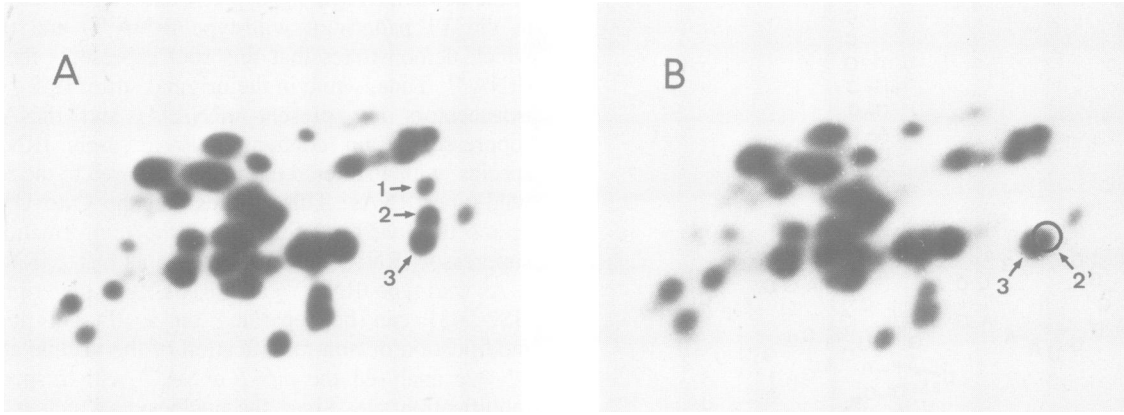

Fig. 5. 2D polyacrylamide gel separation of 3'-end-labelled mitochondrial tRNAs from original strain HS2-5631 (**A**) and suppressor strain SEG15 (**B**). 20 μg of bulk mitochondrial tRNA from each strain was labelled with [α-^{32}P]ATP, in the presence of yeast cytoplasmic tRNA nucleotidyltransferase (Sibler *et al.*, 1983) and separated on a 10% polyacrylamide−4 M urea gel in the first dimension, and on a 20% polyacrylamide gel−4 M urea in the second dimension (Martin *et al.*, 1977). The different tRNA$^{Ser}_{(UCN)}$ spots and the tRNA^{Leu} spot are indicated by arrows: (**1**) tRNA$^{Ser}_2$; (**2**) tRNA$^{Ser}_3$; (**3**) tRNA^{Leu}; (**2'**) suppressor tRNA^{Ser}.

Aminoacylation of tRNA$^{Ser}_{(UCN)}$ in wild-type and suppressor strain

To analyse whether the changes in structure (caused by the C to U mutation at position 42) and in base modification (lack of pseudouridine at position 27) affect the amino-acylation parameters of the *mfs-1* tRNA^{Ser}, acylation kinetics with [^{3}H]serine were performed using a preparation of *E.coli* aminoacyl-tRNA synthetases. *E.coli* enzymes were used instead of yeast mitochondrial enzymes since it has been shown that bacterial enzymes specifically aminoacylate the yeast mitochondrial tRNA$^{Ser}_{(UCN)}$ isoacceptors (Baldacci *et al.*, 1976). Neither the mitochondrial tRNA$^{Ser}_{(AGY)}$ isoacceptor nor the cytoplasmic tRNA^{Ser} are aminoacylated by the *E.coli* enzyme. As an internal standard for comparing mutant and wild-type tRNAs, parallel reactions were carried out using [^{3}H]tyrosine. The results of these experiments showed that under conditions where both tRNAs charged [^{3}H]tyrosine with equal efficiency, the mutant accepted ~80% of the amount of [^{3}H]serine accepted by the wild-type (data not shown). Thus, aminoacylation of the suppressor tRNA^{Ser} is not drastically affected.

Discussion

The results presented here document the discovery of a novel type of +1 frameshift suppressor tRNA. In addition, this is the first reported case of a mitochondrial suppressor tRNA. The suppressor mutation *mfs-1* in *S.cerevisiae* strain SEG15 is located on the mitochondrial genome in the gene coding for tRNA$^{Ser}_{(UCN)}$. The *mfs-1* mutation was shown to suppress specifically a frameshift mutation (M5631) within the mitochondrial *oxi*1 gene coding for subunit II of cytochrome *c* oxidase (Weiss-Brummer *et al.*, 1989). Preliminary analyses of *in vivo* labelled mitochondrial translation products indicate that the suppressor strain produces ~20% more subunit II protein than the original strain (Hüttenhofer *et al.*, unpublished results). This suggests that the *mfs-1* tRNA^{Ser} is a rather strong suppressor.

The mutational change within the tRNA$^{Ser}_{(UCN)}$ gene is a transition from C to T at position 42. This change affects the secondary structure of the tRNA by converting a stable base pair (G28−C42) in the anticodon stem to a weaker pair

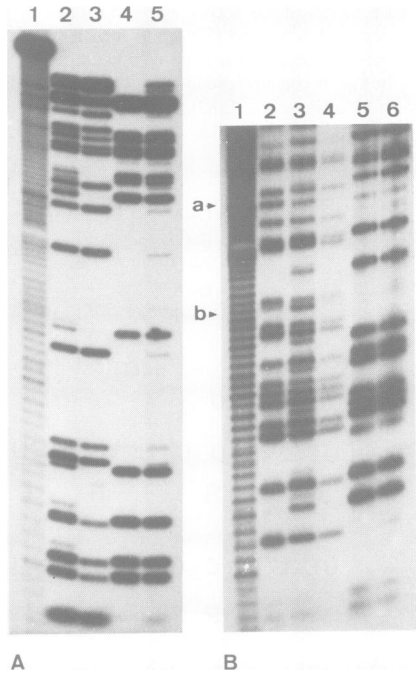

Fig. 6. RNA sequence analysis of suppressor and wild-type tRNAs. (A) Sequencing gel of partial T1 RNase digests. **Lane 1**, sequencing ladder; **lane 2**, *mfs-1* tRNA^{Ser} of strain SEG15; **lane 3**, tRNA$^{Ser}_3$ of strain HS25631; **lane 4**, tRNA^{Leu} of strain HS2-5631; **lane 5**, tRNA^{Leu} of strain SEG15. (B) Chemical sequencing (U reaction). **Lane 1**, sequencing ladder; **Lane 2**, tRNA$^{Ser}_3$ of strain HS2-5631; **lane 3**, *mfs-1* tRNA^{Ser} of strain SEG15; **lane 4**, tRNA$^{Ser}_2$ of strain HS2-5631; **lane 5**, tRNA^{Leu} of strain HS2-5631; **lane 6**, tRNA^{Leu} of strain SEG15. Arrowhead (a) indicates a band corresponding to the unmodified U residue at position 27 in tRNA$^{Ser}_3$ and in *mfs-1* tRNA^{Ser} (**lanes 2** and **3** respectively). This band is absent in tRNA$^{Ser}_2$ (**lane 4**) due to the presence of pseudouridine (Ψ) which is not cleaved by the chemical U reaction. Arrowhead (b) indicates the mutational site C → U at position 42 in *mfs-1* tRNA^{Ser}.

(G28−U42). Until recently, all +1 frameshift suppressor tRNAs in prokaryotes and eukaryotes were found to contain an additional nucleotide within the anticodon region thus leading to an 8 nucleotide anticodon loop instead of the 7 nucleotide loop observed in wild-type tRNAs (Riddle and Carbon 1973; Roth, 1981; Bossi and Roth, 1981; Cummins

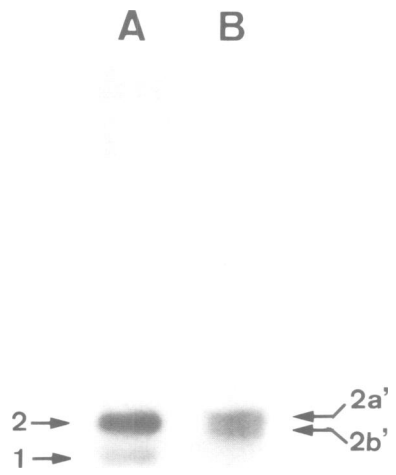

Fig. 7. RNA gel transfer (Northern) hybridization analysis of mitochondrial tRNAs from strain HS2-5631 (**A**) and suppressor strain SEG15 (**B**). Equal amounts of RNA were loaded on a polyacrylamide gel (9.6% acrylamide, 0.4% bisacrylamide) without urea and electrophoresed in the cold room at 10 V/cm. After separation, RNA was transferred on a nylon membrane and hybridized with a nick-translated $tRNA^{Ser}_{(UCN)}$ gene probe (337 bp *Hpa*II fragment). (**A**) arrows (1) and (2) indicate the $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$ bands of strain HS2-5631 respectively; (**B**) arrows (2a') and (2b') indicate the two closely migrating bands of *mfs-1* $tRNA^{Ser}$ in strain SEG15.

et al., 1982; Gaber and Culbertson, 1982; Bossi and Smith, 1984). The presence of an extra anticodon base is sufficient to cause a detectable level of frameshift suppression due to a four base translocation of the mRNA on the ribosome (Gaber and Culbertson, 1984). This indicates that the tRNA anticodon loop size plays an important role in maintenance of the proper reading frame in mRNA. An apparent exception was a yeast proline frameshift suppressor tRNA (*SUF7*) reported by Winey *et al.* (1986), which contains a base alteration in the anticodon stem at position 39. However, as this suppressor mutation disrupts the bottom base pair of the anticodon stem and leads to a 9 nucleotide anticodon loop, this finding does not contradict the previously observed correlation between +1 frameshift suppression and expansion of the anticodon loop size of a frameshift suppressor tRNA. In contrast, very recent studies have demonstrated that frameshift suppression can be accomplished by mutant tRNAs with normal anticodon loop size. First, several missense and nonsense suppressors were found by Tucker *et al.* (1989) to correct +1 frameshift mutations. Particularly notable in that study was a lysine tRNA whose only alteration was a C to U70 base substitution in the amino acid acceptor stem. Second, certain mutant derivatives of $tRNA^{Gly}_2$ of *Salmonella* were selected as suppressors of a −1 frameshift mutation. These included a insertion in the TΨC loop, two different base substitutions in the TΨC stem and a G to A base change at the 5′ end (O'Mahony *et al.*, 1989). Finally, in this paper we have described a novel mitochondrial frameshift suppressor tRNA, *mfs-1* $tRNA^{Ser}$, which has a 7 nucleotide anticodon loop but harbours a mutation affecting the second base pairing of the anticodon stem. The results of all three of these recent studies demonstrate that shifting of the reading frame can be induced by mutant tRNAs with normal anticodon loop size but with a structural alteration in another region of the tRNA molecule, even in one far from the anticodon.

To obtain more detailed information about the mechanism of frameshift by the *mfs-1* $tRNA^{Ser}$, we investigated its transcription, processing, structure and aminoacylation properties. Although there is ample evidence that base substitutions can affect the transcription of tRNA genes (e.g. De Franco *et al.*, 1980; Koski *et al.*, 1980) and the processing of tRNA transcripts (Altman, 1971; McClain *et al.*, 1975), the C to U change in $tRNA^{Ser}_{(UCN)}$ does not appear to affect transcription of the gene or processing of the transcripts, since comparable levels of $tRNA^{Ser}_{(UCN)}$ transcripts are present in the suppressor strain SEG15 and in the original strain HS2-5631; furthermore, the extremities of the *mfs-1* tRNA transcripts are processed normally (as in wild-type). Nevertheless, the mutation in the tRNA anticodon stem does affect the post-transcriptional modification, secondary/tertiary structure and aminoacylation of the *mfs-1* tRNA.

Post-transcriptional modification alteration of mfs-1 $tRNA^{Ser}$

The wild-type allele of the $tRNA^{Ser}_{(UCN)}$ gene was shown to be special in yeast mitochondria in so far as it encodes two isoaccepting tRNA species, i.e. $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$, which differ by only a nucleoside modification at position 27 in the anticodon stem (Martin *et al.*, 1982). At this position, the $tRNA^{Ser}_2$ contains pseudouridine while the $tRNA^{Ser}_3$ carries a non-modified uridine. These two isoacceptors can be separated by chromatography on a RPC5 column and by 2D-PAGE, suggesting that they are likely to adopt different conformations (Martin *et al.*, 1982). In this context, it is interesting that we could show that in the suppressor strain SEG15, only the non-modified form of the tRNA is present. Thus the mutation C42 to U42 in the anticodon stem of *mfs-1* $tRNA^{Ser}$ prevents the modification of the spatially close residue U27. This result agrees with earlier data of Curran and Yarus (1986) showing that mutations in the anticodon stem of an amber suppressor $tRNA^{Trp}$ (*Su7*) alter or eliminate post-transcriptional modifications in the anticodon arm.

Changes in structure and stability of the mfs-1 $tRNA^{Ser}$

The C to U mutation within the anticodon stem of the *mfs-1* $tRNA^{Ser}$ appears to influence the structure and conformational stability of the tRNA molecule. This was deduced from 2D-PAGE analysis of suppressor and wild-type $tRNA^{Ser}$. In fact, the *mfs-1* tRNA revealed a secondary/tertiary structure different from both $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$. This can be concluded from a localization of the spot of *mfs-1* tRNA on the 2D gel different from those of both $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$ of the original strain HS2-5631. With respect to the two wild-type serine isoacceptors, the suppressor tRNA lacks two structural elements which may stabilize the spatial structure of a tRNA molecule. Firstly, the only G−C base pairing in the anticodon stem is replaced by a weaker G−U pairing due to the primary change C to U at position 42; this is expected to destabilize the conformation of the *mfs-1* $tRNA^{Ser}$. Secondly, compared to $tRNA^{Ser}_2$, the suppressor tRNA is unmodified at

position 27; this should further decrease the stability of the spatial structure of the *mfs-1* tRNA, especially since hypomodification is also known to destabilize tRNA conformation (Björk *et al.*, 1987). Thus compared to the two wild-type isoacceptors, the suppressor tRNA should exhibit a lower stability, the decreasing order of stability being $tRNA^{Ser}_2 > tRNA^{Ser}_3 >$ *mfs-1* $tRNA^{Ser}$.

Acylation properties of the mfs-1 tRNA^{Ser}

Besides the changes in post-transcriptional modification and conformation of the *mfs-1* $tRNA^{Ser}$, its charging ability seems to be slightly affected, since the efficiency of *in vitro* aminoacylation of SEG15 $tRNA^{Ser}_{(UCN)}$ is reduced to ~80% of the HS2-5631 $tRNA^{Ser}_{(UCN)}$ aminoacylation efficiency. Björk and Kjellin-Straby (1977) showed that the lack of modification of a G residue at position 26 in yeast cytoplasmic $tRNA^{Ser}$ reduces the *in vitro* charging ability by ~20%. In that context, the lack of modification of U27 in the *mfs-1* tRNA could directly affect the efficiency of aminoacylation of this tRNA. However, we do not think that a reduction by 20% in aminoacylation leads to a severe shortage of acylated $tRNA^{Ser}_{(UCN)}$ in mitochondria of suppressor strain SEG15. Although Weiss and Gallant (1983) showed that frameshifting is increased due to limitation of a tRNA species, it seems less plausible to us that the suppressor effect of the *mfs-1* tRNA results from that mechanism. We favour the idea that a structural change in the suppressor tRNA caused by the alteration of a G–C base pairing and the lack of U27 modification in the anticodon stem is much more important for the suppressor function (see below).

Models for frameshift suppression by the mfs-1 tRNA^{Ser}

The base substitution C42 to U42 in the mitochondrial frameshift suppressor tRNA has a destabilizing effect on the anticodon stem structure; this might lead to a conformational change in the anticodon loop. That some structural connection exists between anticodon stem and loop has been demonstrated by Seong and RajBhandary (1987) in the case of *E.coli* $tRNA^{fMet}_2$: base substitutions within the anticodon stem of this tRNA alter the accessibility of anticodon loop residues. Therefore, the alteration in the anticodon stem of the *mfs-1* tRNA may affect translocation by altering some structural feature of the anticodon region such as base stacking (Curran and Yarus, 1987).

Based on these considerations, we propose the following model for the mechanism of frameshift suppression by the *mfs-1* tRNA. The perturbed anticodon stem structure might influence the stacking of bases in the anticodon loop so as to allow the residue U33 to form a Watson–Crick pairing with the A residue 3′ adjacent to the serine codon UCC at the mutational site in the *oxi1* mRNA (Figure 4). This would favour translocation of a four base codon UCCA on the ribosome thus restoring the wild-type reading frame. Thus, the +1 frameshift mutation M5631 could be suppressed by a 'four base reading' of *mfs-1* $tRNA^{Ser}$. However, such a four base reading in a 7 nucleotide anticodon loop has been found to be improbable or non-existent in *E.coli* (Ayer and Yarus, 1986), but it remains possible that the yeast mitochondrial decoding mechanism is different from that of *E.coli*. Alternatively, the structural changes in the anticodon stem of the *mfs-1* $tRNA^{Ser}$ (C42 to U42 mutation and lack of modification of residue U27) might destablilise the bottom base pair (A-Ψ) of this stem in some fraction of the molecules. This would lead to a 9 nucleotide anticodon loop able to read a four base codon, as is the case of the yeast *SUF7* frameshift suppressor tRNA (Winey *et al.*, 1986).

A Watson–Crick pairing of anticodon loop residue U33 with position four of a codon quadruplet is, however, not a prerequisite for a four base translocation mechanism to occur. Indeed, Gaber and Culbertson (1984) showed that codon recognition by a +1 frameshift suppressor tRNA does not necessarily require position four base pair, although the stability of position four codon–anticodon interaction influences the efficiency of suppression. Therefore, just a sterical protection of the A base of the four base codon UCCA by the *mfs-1* $tRNA^{Ser}$ anticodon region could suffice to prevent this base from being read by another tRNA.

Frameshift suppression due to a mutation within the tRNA anticodon stem is a feature that up to now seems unique to the *mfs-1* tRNA. Curran and Yarus (1986) performed an extensive study of mutations within the anticodon stem of the amber suppressor $tRNA^{Trp}$ (*Su7*) and searched for evidence that such mutations could induce frameshifting. Surprisingly, none of the base alterations studied within this tRNA anticodon stem decreased the accuracy of reading frame maintenance. The authors concluded that the sequence and conformation of the anticodon stem are not critical in determining the length of translocation. Although our mutant stands in contrast to their conclusion, the data do not necessarily conflict. Firstly, the *Su7* and *mfs-1* tRNA have totally different anticodon stem structures. While in the *Su7* tRNA this stem comprises four G–C and one A–U pair, it contains four A–U and one G–U pair in the mitochondrial suppressor tRNA. This indicates a reduced stability of the *mfs-1* tRNA anticodon stem compared to that of the *Su7* tRNA. Thus, alteration of a base pairing in the *Su7* anticodon stem might have a less dramatic effect on the conformation of anticodon loop residues than in the mitochondrial suppressor tRNA. Secondly, if one assumes that a 'four base reading' mechanism accounts for the suppressor effect of the *mfs-1* $tRNA^{Ser}$, a stable pairing may be formed between anticodon loop residue U33 and the A base of the UCCA quadruplet. In the case of the *Su7* tRNA mutants, the same would demand a U33–C non-standard pairing which might be less conducive to frameshifting. Finally, Curran and Yarus (1986) opted not to examine the effect of G–U base pairs in the anticodon stem of the *Su7* tRNA.

An alternative model for frameshift suppression by the *mfs-1* $tRNA^{Ser}$ is the following. The *mfs-1* mutation might be a Hirsh suppressor-like alteration, which has recently been shown to act quite generally, enhancing coding by a tRNA with virtually any other defect (Smith and Yarus, 1989). The changes within the anticodon stem of the *mfs-1* tRNA are in spatial proximity to the Hirsh (D-arm) mutation, being localized in the same stacked unit of the tRNA tertiary structure. The effect of the changes would be to allow coding by the suppressor tRNA in a conformation that blocked the next codon, and therefore forced reading in the +1 frame. This model, however, implies that *mfs-1* should also suppress other mitochondrial mutations, a conclusion which contradicts previously published results (Weiss-Brummer *et al.*, 1989).

'To shift or not to shift . . '

Wild-type $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$ both encoded by the same single copy gene, are the only UCN reading serine-tRNAs in mitochondria and hence are indispensable for mitochondrial protein synthesis (Martin *et al.*, 1982). As the suppressor strain SEG15 seems to contain only one UCN reading $tRNA^{Ser}$ (i.e. *mfs-1* tRNA), this highlights an interesting feature of the *mfs-1* $tRNA^{Ser}$: besides suppressing a +1 frameshift, the mutant tRNA must still be able to read UCN codons—without causing a frameshift—sufficiently well for the cell to grow. A similar situation, recently described by O'Mahony *et al.* (1989), is encountered in the case of several −1 frameshift suppressors derived from $tRNA^{Gly}_2$ of *S.typhimurium*. Besides causing −1 frameshifting, these mutant tRNAs must still be able to decode their normal codon GGA. The authors proposed that these two decoding abilities could be achieved by two conformations of each tRNA. Likewise, two interconvertible tRNA conformations of the *mfs-1* mutant could exist, one for preferentially inducing frameshift and one for reading UCN codons without causing a frameshift. In fact, there are hints that two conformations of $tRNA^{Ser}_{(UCN)}$ may exist in suppressor strain SEG15, because two mutant $tRNA^{Ser}_{(UCN)}$ transcripts can be separated on a non-denaturing polyacrylamide gel. Although extensive structural investigations of the *mfs-1* tRNA need to be performed, it is plausible that these two transcripts resemble two conformations of different decoding abilities.

Materials and methods

Strains

Genotypes and origin of strains used in this study are given in Table I. Culture media and growth conditions of the yeast strains have been described previously (Weiss-Brummer *et al.*, 1989).

Isolation of mitochondrial DNA

Yeast cells were grown at 30°C to early stationary phase in a medium containing 4% (w/v) glucose and 1% (w/v) yeast extract. Mitochondria were prepared from protoplasts obtained by treatment of the cells with 0.3 mg/g cells of zymolyase 20 000 (Seikagaku Kogyo Co., Ltd., Tokyo, Japan). Mitochondrial DNA was extracted with 0.5% SDS and purified by centrifugation in CsCl gradients (Hudspeth *et al.*, 1980).

DNA sequencing

A 2000 bp *Hae*III restriction fragment was cloned into the *Sma*I site of M13mp8 (Messing *et al.*, 1981). The fragments of the original strain HS2-5631 and of suppressor strain SEG15 were sequenced on both strands by the dideoxy chain termination method using synthetic oligonucleotides as primers (Sanger *et al.*, 1977, 1980).

Mitochondrial RNA isolation and analysis

Mitochondria were prepared as described above. Mitochondrial RNA was extracted by the method of Locker (1979) and was electrophoresed either on a 10% polyacrylamide−8 M urea gel or on a 10% polyacrylamide gel without urea. The RNA was transferred to Zeta probe membrane (Bio-Rad) by electroblotting (Alwine *et al.*, 1977) and hybridized with a nick-translated $tRNA^{Ser}_{(UCN)}$ gene probe (337 bp *Hpa*II fragment). Bulk mitochondria tRNA was prepared by DEAE−cellulose column chromatography, 3'-end-labelled with tRNA nucleotidyltransferase and separated by 2D-PAGE (Martin *et al.*, 1977).

S1 nuclease mapping of both 5' and 3' termini of $tRNA^{Ser}_{(UCN)}$ transcripts in the original mutant and in suppressor strain SEG15 was performed as previoulsy described (Bordonné *et al.*, 1987). The protected fragments were electrophoresed on an 8% polyacrylamide sequencing gel along with four enzymatic sequencing reactions of M13 ssDNA for determination of the exact length of the protected fragments.

Aminoacylation of tRNA

Mitochondrial tRNA (0.05−1 mg/ml) was acylated *in vitro* with [^{3}H]serine and [^{3}H]tyrosine (CEA, Saclay, France) using a 3.5 μg *E.coli* aminoacyl-tRNA synthetases (Muench and Berg, 1966). Acylations were performed in 100 mM Tris−HCl pH 8, 10 mM ATP, 25 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 0.6 mg/ml glutathione, 50 μM [^{3}H]amino acid. After incubation at 37°C, trichloroacetic acid precipitable counts were determined.

RNA sequencing

Chemical sequencing of tRNA isolated by 2D-PAGE was performed as described by Peattie (1987). Enzymatic sequencing by limited T1 RNase digestion was performed according to Donis-Keller *et al.* (1977).

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