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Yeast mitochondrial $tRNA^{Ile}$ and $tRNA_m^{Met}$: nucleotide sequence and codon recognition patterns

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ABSTRACT

The nucleotide sequence of yeast mitochondrial isoleucine- and methionine-elongator tRNA have been determined. Interestingly, long stretches of almost identical nucleotide sequences are found within these two tRNAs and also within the yeast mt $tRNA_f^{Met}$, suggesting that the 3 tRNAs may have arisen from a common ancestor. Both mt $tRNA_m^{Met}$ and $tRNA^{Ile}$ contain all the structural characteristics which are present in the standard cloverleaf, except that the mt $tRNA_m^{Met}$ contains an extra unpaired nucleotide within the base-paired TψC stem. This rather unusual feature may have an influence on the decoding properties of the C-A-U anticodon of mt $tRNA_m^{Met}$ by confering the ability to translate not only the codon A-U-G but also A-U-A.

INTRODUCTION

The genetic code used in mitochondria not only differs from the standard code but it also varies from one organism to another. For example, usage of the codon A-U-A depends on the origin of mitochondria. In **Neurospora crassa** (1) and **Aspergillus nidulans** (2) mitochondria, A-U-A specifies isoleucine following the universal code, whereas in mammalian mitochondria A-U-A codes for methionine (3). It has been suggested that A-U-A also codes for methionine in yeast mitochondria based on comparison of the DNA sequence of the ribosomal protein gene var1 with the amino acid composition of the purified protein (4). In order to gain an insight into the molecular basis of A-U-A recognition in yeast mitochondria, we have purified the mitochondrial (mt) methionine elongator- and isoleucine tRNA and determined their nucleotide sequence.

METHODS

Crude mt tRNA of **Saccharomyces cerevisiae** IL8-8C was fractionated by RPC-5 column chromatography (5). The fractions showing acceptor activity for [^{3}H]-methionine and [^{3}H]-isoleucine using a mitochondrial enzyme extract were pooled separately and further purified by two-dimensional poly-

acrylamide gel electrophoresis (6).

The procedures used for sequence analysis of tRNA by postlabeling techniques were the same as described previously (7,8).

RESULTS AND DISCUSSION

Only one mt $tRNA^{Ile}$ species and two isoacceptors of mt $tRNA^{Met}$ have been detected in yeast mitochondria by either RPC-5 column chromatography or two-dimensional gel electrophoresis (5,6,9). The two methionine isoacceptors (e.g. $tRNA_1^{Met}$ and $tRNA_2^{Met}$) have been shown to correspond to $tRNA_m^{Met}$ and $tRNA_f^{Met}$, respectively (5). The primary structure of the mt $tRNA_f^{Met}$ has already been reported (10). The pure mt $tRNA^{Ile}$ and $tRNA_m^{Met}$ were obtained by two-dimensional polyacrylamide gel electrophoresis of RPC-5 column chromatographic fractions enriched in these two tRNAs (see fig. 1 in ref. 9).

Most of the nucleotide sequence of either mt $tRNA^{Ile}$ or mt $tRNA_m^{Met}$ were determined using the method of Stanley and Vassilenko (11), with the modifications reported in ref. (7). All residues suspected of being modified were identified by two-dimensional thin-layer chromatography in different solvent systems (7). To confirm the nucleotide assignments and to determine

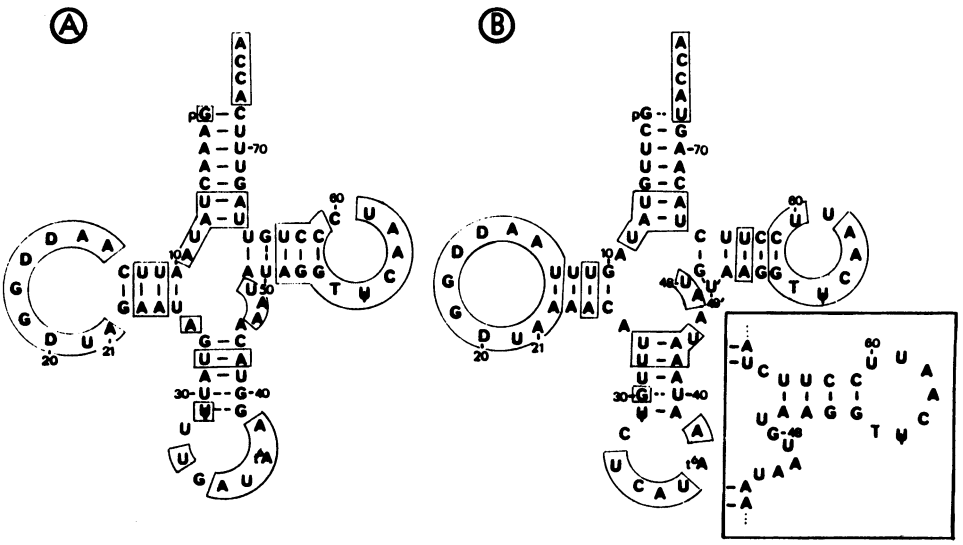

FIGURE 1 - Cloverleaf structures of the yeast mitochondrial $tRNA^{Ile}$ (A) and $tRNA_m^{Met}$ (B). Residues in common between mt $tRNA^{Ile}$ and mt $tRNA_m^{Met}$ are boxed in (A) and those in common between mt $tRNA_m^{Met}$ and mt $tRNA_f^{Met}$ (10) are boxed in (B). The inset in (B) shows an alternative representation of the extra-arm and TψC arm regions of mt $tRNA_m^{Met}$.

5'- and 3'-end sequences, read-off sequencing gels were run on [^{32}P]-end-labeled samples. The complete nucleotide sequence of the yeast mt $tRNA^{Ile}$ and mt $tRNA_m^{Met}$, shown in fig. 1, were deduced from the resulting data. Finally, the RNA sequences obtained here were found to be in total agreement with the determined DNA sequence of their respective genes (12 ; R. Bordonné **et al.**, unpublished results).

Both the mt $tRNA^{Ile}$ (76 nucleotides long ; fig. 1A) and the mt $tRNA_m^{Met}$ (77 nucleotides long ; fig. 1B) contain 7 modified residues, which occur at the same positions in their respective cloverleaf structures : D_{16}, D_{17}, D_{20}, Ψ_{31}, t^6A_{37}, T_{54} and Ψ_{55}. Interestingly these modified nucleosides are present in regions of common primary sequence in the two tRNAs. It should also be emphasized that, in terms of overall sequence homology, the mt $tRNA_m^{Met}$ is as homologous to the mt $tRNA^{Ile}$ (48 common residues ; see fig. 1A) as it is to the mt $tRNA_f^{Met}$ (10) (46 common residues ; see fig. 1B). Three long stretches of common sequences (residues 6-24, anticodon region and residues 51-63) are almost perfectly conserved in these 3 mt tRNAs suggesting that they may have arisen by gene duplication from a common ancestral tRNA.

The secondary structure of mt $tRNA_m^{Met}$ (fig. 1B) differs from the one deduced by Corruzzi **et al.** (12), although the two models were built starting from the same basic primary structure. In Corruzzi **et al.** (12), the representation of the extra-arm and the TψC arm (see inset of fig. 1B) is unusual in that it shows an extra-loop of 6 residues (with a G in position 48), and an unpaired C residue between the TψC arm and the acceptor stem leaving a TψC stem with only 4 base-pairs. The model we propose has an extra-loop of 4 residues (with a U in position 48) and the TψC stem has 5 base-pairs but one extra unpaired nucleotide (residue $U_{49'}$) bulges out of this stem (fig. 1B). We prefer this representation since it shows fewer exceptions to the standard cloverleaf and allows a tertiary interaction between A_{15} and U_{48}. Moreover, a nucleotide bulging out of a stem of the tRNA cloverleaf - referred to as an outloop by Francis and Dudock (13) - has also been found in 4 other tRNA structures. In the yeast mt $tRNA^{Phe}$ (14) and mt $tRNA_2^{Lys}$ (9,15), the outloop also occurs in the TψC stem but between residues 50 and 51, whereas in the spinach chloroplast $tRNA_1^{Ile}$ (13) it occurs in the anticodon stem. Finally, in the highly unusual bovine liver minor $tRNA^{Ser}$ (16), the only non-organellar tRNA known to have an outloop, this feature is found in the TψC stem but on its 3'-side.

Aside from this very unusual feature of mt $tRNA_m^{Met}$ which may influence

its codon recognition pattern (for further discussion, see below), both mt $tRNA_m^{Met}$ and mt $tRNA^{Ile}$ contain all of the structural characteristics, including invariant and semi-invariant residues, present in the standard cloverleaf (17).

The mt $tRNA^{Ile}$ has an anticodon G-A-U for translation of the A-U-C and A-U-U isoleucine codons and the mt $tRNA_m^{Met}$ has an anticodon C-A-U for decoding A-U-G. Thus, if only the respective anticodon sequences are considered, neither mt $tRNA^{Ile}$ nor mt $tRNA_m^{Met}$ should be able to recognize the A-U-A codon. However, in view of the results obtained by Hudspeth **et al.** (4), A-U-A is most probably a codon for methionine rather than isoleucine in yeast mitochondria. Since there are only two methionine isoacceptors (e.g. mt $tRNA_m^{Met}$ and $tRNA_f^{Met}$) in yeast mt tRNA preparations (see above), the A-U-A decoding species must be the mt $tRNA_m^{Met}$. Preliminary results of **in vitro** protein synthesis in an **E.coli** S100 extract using TMV mRNA, indicate that the mt $tRNA_m^{Met}$ inserts methionine into the TMV coat protein in response to A-U-A (A.P. Sibler, unpublished results).

One may wonder how the C-A-U anticodon of mt $tRNA_m^{Met}$ could recognize the A-U-A codon. By developing molecular models, Mikelsaar concluded that an H-bonded C:A pair in the wobble position of the codon-anticodon complex is possible, but in the trans configuration (18). This pairing is accompanied by assymetrical conformational changes, the main rearrangements occurring on the adenosine of the codon. Furthermore, modification of the C residue in the wobble position of the anticodon may either favor or reduce the stability of the C:A pair. For example, pairing of m^5C with A is possible whereas ac^4C:A and Cm:A pairs are unstable (18). The A-U-A specific $tRNAs^{Ile}$ of **E.coli** (19) and bacteriophage T_4 (20) contain a C derivative of unknown structure (C*) in the wobble position, which restricts the C*:G wobble while allowing C*:A wobble. An unknown C derivative is also present in the wobble position of the spinach chloroplast A-U-A decoding $tRNA^{Ile}$ (13). In contrast, in the yeast mt $tRNA_m^{Met}$ the C residue of the C-A-U anticodon is unmodified. Structural features outside of the anticodon are also known to influence the codon reading properties of a C in the wobble position. For example, in the case of the **E.coli** opal suppressor $tRNA^{Trp}$ (anticodon C-C-A), a sequence alteration in the dihydrouridine stem confers the ability to translate the U-G-A nonsense codon (21). It is tempting to speculate that the extra unpaired nucleotide in the TψC stem of yeast mt $tRNA_m^{Met}$ similarly enhances C:A wobble, thus permitting the decoding of both A-U-A and A-U-G. A

similar functional role for the outloop in the anticodon stem of spinach chloroplast $tRNA_1^{Ile}$ has also recently been proposed (13).

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