

**Nucleotide sequence of bean mitochondrial tRNA<sup>Leu4</sup> and of its cytoplasmic counterpart. Re-examination of the modified nucleotide present at position 12 in bean mitochondrial and cytoplasmic tRNA<sup>Leu1</sup> sequences**

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We reported recently that four *Phaseolus vulgaris* mitochondrial (mt) tRNA<sup>Leu</sup> species are nuclear-encoded and imported from the cytoplasm into the mitochondria (1). Here, we present the sequence of one of them namely tRNA<sup>Leu4</sup>, isolated according to purification procedures described in 1. Its sequence, determined as previously described (1), is shown on fig. 1. In order to purify the corresponding cytoplasmic (cyt) tRNA<sup>Leu</sup>, a 5'-end labeled oligonucleotide (2), corresponding to mt tRNA<sup>Leu4</sup> complementary sequence (from position 39 to position 50), was used as a probe to screen total bean cytoplasmic tRNA fractionated on RPC-5 column. Fractions showing positive hybridization were checked for leucine accepting activity (fig. 2) and further submitted to polyacrylamide gel electrophoresis under denaturing conditions (3). The cyt tRNA<sup>Leu</sup> sequence was then determined and found to be identical to mt tRNA<sup>Leu4</sup> except for one post-transcriptional modification occurring at position 18 (Gm in mt tRNA<sup>Leu4</sup> instead of G in cyt tRNA<sup>Leu4</sup>). Similar results were previously reported in the case of bean mt and cyt tRNA<sup>Leu1</sup> and tRNA<sup>Leu2</sup> species, but whether this post-transcriptional modification occurs in the cytoplasm or in the mitochondria remains to be established.

Both bean mt and cyt tRNA<sup>Leu4</sup> species are 86 nucleotides long. They contain an unknown modified nucleotide (N) at the wobble position of the anticodon. They show 62.6 and 70% of sequence homology with bean mt and cyt tRNAs<sup>Leu1</sup> and tRNAs<sup>Leu2</sup> respectively. As bean mt and cyt tRNAs<sup>Leu2</sup> and tRNAs<sup>Leu4</sup> have ac<sup>4</sup>C at position 12, we re-examined the sequence of bean mt and cyt tRNAs<sup>Leu1</sup> species where m<sup>5</sup>C was described at this position (3). In these two tRNAs<sup>Leu</sup> ac<sup>4</sup>C is also present (and not m<sup>5</sup>C), so that mt and cyt tRNAs<sup>Leu1</sup>, tRNAs<sup>Leu2</sup> and tRNAs<sup>Leu4</sup> all have ac<sup>4</sup>C at this position.

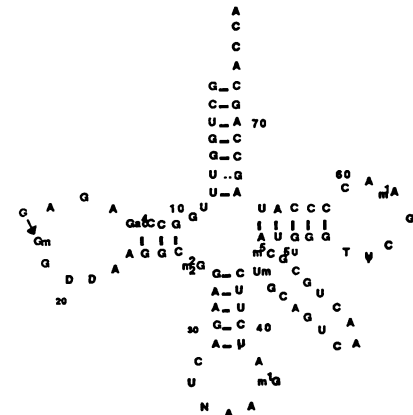


Fig. 1. Nucleotide sequences of bean mt tRNA<sup>Leu4</sup> and of its cytoplasmic counterpart. Cloverleaf represents the structure of mt tRNA<sup>Leu4</sup>. Arrow indicates the difference between these two tRNAs<sup>Leu</sup>. N(34) : unidentified modified nucleotide.

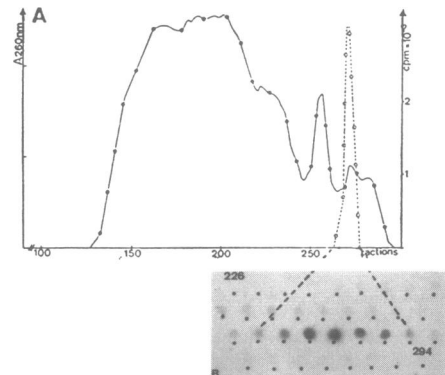


Fig. 2. A) RPC-5 fractionation of bean total cyt tRNA. 98 mg of cyt tRNA in 0.01 M sodium acetate buffer pH 4.7 containing 0.01 M MgCl<sub>2</sub> and 0.3 M NaCl were loaded on the column (180x2.5 cm). Elution was performed in the same buffer with a linear gradient (2x2.5 l) from 0.33 to 0.73 M NaCl. Fractions of 15 ml were collected. (—) : A<sub>260</sub> nm; (---) : <sup>3</sup>H-leucine accepting activity tested on the fractions showing positive hybridization (see B). B) tRNA dot blot hybridization with labeled oligonucleotide specific for mt tRNA<sup>Leu4</sup>.

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**References**

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