Sequence and codon recognition of bean mitochondria and chloroplast tRNAs^{Trp}: evidence for a high degree of homology

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ABSTRACT

Bean mitochondria and chloroplast tRNAs^{Trp}, purified by RPC-5 chromatography and two-dimensional gel electrophoresis, have been sequenced using post-labeling techniques. The high degree of sequence homology between bean mitochondria and chloroplast tRNAs^{Trp} shows that these two tRNAs are coded for by closely related genes which have probably evolved from a common ancestor gene. The anticodon of bean mitochondria tRNA^{Trp} is CmCA, which can recognize UGG (the codon for tryptophan in the universal code) and is complementary neither to UGA (which codes for tryptophan in mammalian and yeast mitochondria) nor to CGG (which could be a tryptophan codeword in plant mitochondria).

INTRODUCTION

So far little information is available on plant mitochondrial (mt) tRNAs, and only one tRNA (bean mt tRNA^{Phe}) has been sequenced ; it shows 76 % sequence homology with its chloroplast (cp) counterpart (1). On the other hand, the sequences of five mt tRNA genes have been reported : one tRNA^{Met} gene in wheat (2), two in maize (3), one in Oenothera (4) and one tRNA^{His} gene in maize (5). Whereas, one of the two maize mt tRNA^{Met} genes (coding probably for the elongator tRNA^{Met}) shows only 45,4 % homology with its cp counterpart (3), the other maize mt tRNA^{Met} gene, the wheat and Oenothera mt tRNA^{Met} genes (which are probably coding for the initiator tRNA^{Met}) show a much higher degree of homology with the corresponding cp tRNA or tRNA gene (71-78 %) and there is 100 % homology between the maize cp and mt tRNA^{His} genes.

But the determination of the sequence of a tRNA gene present in the mitochondrial DNA does not tell whether this gene is actually transcribed into a functional tRNA. It has been reported that chloroplast DNA sequences are found in mitochondrial DNA (for a review see ref. 6), but one does not know if these sequences are transcribed, so that the characterization of a mt tRNA requires the determination of its nucleotide sequence.

Studies on plant mt tRNA^{Trp} are of special interest, since it has been proposed that, in plant mitochondria, tryptophan could be coded not only by the universal codon UGG, but also by CGG, which normally codes for arginine (7-9).

We have sequenced bean mt tRNA^{Trp} and have found that it has a high degree of homology with spinach cp tRNA^{Trp} which had been previously sequenced in our laboratory (10). We have therefore also sequenced bean cp tRNA^{Trp}, in order to compare it to bean mt tRNA^{Trp}. We report here that bean mt and cp tRNAs^{Trp} have a very high degree of sequence homology and we discuss the coding properties of the bean mt tRNA^{Trp} which has been sequenced.

MATERIAL AND METHODS

Total mt tRNA was prepared from mitochondria isolated from dark-grown bean (**Phaseolus vulgaris**) hypocotyls, as previously described (11) and fractionated on a RPC-5 column (12) using a NaCl gradient from 0.5 M to 1.1 M in 0.01 M Na acetate buffer, pH 4.7, containing 0.01 M MgCl$_2$. Mitochondrial tRNA^{Trp} was identified by aminoacylation, using either **E.coli** (13) or wheat germ aminoacyl-tRNA synthetases and ^{3}H-tryptophan. Fractions containing mt tRNA^{Trp} were pooled, concentrated and further subjected to two-dimensional polyacrylamide gel electrophoresis (14). One of the 12 spots separated on the gel was found by aminoacylation to contain a tRNA^{Trp}. From 16 kg of dark-grown bean hypocotyls, 2 µg of pure mt tRNA^{Trp} were obtained.

Bean cp tRNA^{Trp} was purified from bean leaves, as described for spinach cp tRNA^{Trp} (10).

The nucleotide sequences of bean mt tRNA^{Trp} and cp tRNA^{Trp} were determined using post-labeling techniques (15) and approaches previously described (16). Most of the data were obtained using the Stanley and Vassilenko technique (17), but a number of fragments were also sequenced using mobility shift analysis (15).

Labeling of tRNA^{Trp} at the 3' end and hybridization of mt tRNA^{Trp} with mt DNA were carried out as previously described (1).

RESULTS AND DISCUSSION

The structures of bean mt and cp tRNAs^{Trp} are shown in figure 1, with the differences indicated by arrows.

If one does not take into account post-transcriptional modifications, there are three (or possibly four) differences between mt and cp tRNA^{Trp} : G
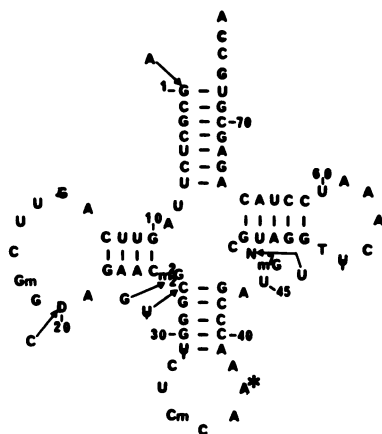
Fig. 1 Nucleotide sequences of bean mitochondrial and chloroplast tRNAs[Trp].
The cloverleaf represents the structure of bean mt tRNA[Trp], and the
nucleotides which differ in bean cp tRNA[Trp] are indicated by arrows.

A* (37) = i^6A or ms^2i^6A
N (47) = unidentified modified nucleotide

at position 1 in mt tRNA[Trp] is replaced by A in cp tRNA[Trp], D at position 20
is replaced by C, and C at position 27 is replaced by ψ. Furthermore, in mt
tRNA[Trp] there is an unidentified modified nucleotide (called N) at position
47, whereas there is a U in cp tRNA[Trp]. However there is a possibility that
N is a modified U : this could be checked either by identifying N (but it
would require large amounts of mt tRNA[Trp]), or by sequencing the
corresponding gene. Depending whether one considers that there are 3 or
4 differences, the percentage of homology between bean mt and cp tRNA[Trp] is
97.3 or 96.2.

In addition, there is a difference at the level of post-transcriptional
modification: bean mt tRNA[Trp] contains m^2G at position 26, whereas G is
found in bean cp tRNA[Trp].

Considering the high degree of homology between bean mt tRNA[Trp] and its
chloroplastic counterpart, we have carefully checked the absence of cp tRNAs
in our preparation of mt tRNAs. Using E.coli enzymes, which are able to
aminoacylate only bean cp tRNAs[Leu] but none of the bean mitochondrial or
cytoplasmic tRNAs [Leu] (18), we did not detect any cp tRNA[Leu] in our mt tRNA
preparations. Furthermore, previous studies on mt tRNAs prepared using the
same method (11) have shown that mt tRNA preparations are not contaminated

by cp tRNA^{Leu}, cp tRNA^{Met}, cp tRNA^{Pro}, cp tRNA^{Lys}, cp tRNA^{Phe}, cp tRNA^{Tyr} or cp tRNA^{Trp} (for reviews, see ref. 13, 19).

Using 3' end-labeled bean mt tRNA^{Trp} as a probe, hybridization was obtained with genomic mt DNA of maize and with cosmid clones of wheat mt DNA. As previous studies in our laboratory have already shown that bean cp tRNA^{Trp} is coded for by the bean chloroplast genome (20), it is clear that bean mt tRNA^{Trp} (isolated from purified mitochondria) and bean cp tRNA^{Trp}, whose sequences have now been determined and are slightly different, are coded for by distinct but very similar genes which may have derived from a common ancestor gene. Considering the rather high degree of homology (76 %) found between bean mt and cp tRNAs^{Phe} (1), the two corresponding genes may also have evolved from a common ancestor gene and this might be true for other plant mt and cp tRNA genes. However more sequence comparisons between mt tRNAs and cp tRNAs would be necessary to draw a general conclusion. In particular it would be very interesting to see whether the maize mt tRNA^{His} gene (5) which shows 100 % homology with its chloroplast counterpart is indeed transcribed into a functional tRNA^{His} in the mitochondria.

Table I shows the percentage of homology between bean mt tRNA^{Trp} and various sequenced tRNAs^{Trp} or tRNA^{Trp} genes. It is interesting to note the

Table I :

Percentage of sequence homology between bean mitochondrial tRNA^{Trp} and various sequenced tRNAs^{Trp} or tRNA^{Trp} genes

Tobacco chloro	98.7
Spinach chloro	97.4
Euglena gracilis chloro	87.0
Escherichia coli su$^+$ UGA	68.8
Escherichia coli	67.5
Dictyostelium discoides	55.8
Wheat Germ cyto	52.6
Yeast cyto	51.9
Bovine liver cyto	50.6
Aspergillus nidulans mito	50.6
Chicken cells cyto	49.3
Neurospora crassa mito	49.3
Yeast mito	48.0
Halobacterium volcanii	48.0
Mouse mito	41.6
Bovine mito	40.3
Rat mito	37.7
Human mito	37.7
Bovine liver mito	35.1

All these tRNA and tRNA gene sequences can be found in ref. 21 except those of wheat germ cyto tRNA^{Trp} (22) and tobacco chloro tRNA^{Trp} (25).

high degree of homology between bean mt tRNA^{Trp} on one hand and prokaryotic and chloroplast tRNAs^{Trp} on the other hand, and in contrast the low degree of homology with the other mt tRNAs^{Trp} or mt tRNA^{Trp} genes sequenced (from mammalian and fungal mitochondria). Similar observations have also been made in the case of bean mt tRNA^{Phe} (1), suggesting that plant mt tRNAs represent a special class among mt tRNAs.

The anticodon of bean mt tRNA^{Trp}, CmCA can recognize the usual tryptophan codon UGG and, theoretically, should not be able to read CGG, a codon which normally specifies arginine but which has been postulated to code for tryptophan in maize, Oenothera and wheat mitochondria (7-9). But the possibility cannot be ruled out that another mt tRNA^{Trp}, with a CCG anticodon, exists in plant mitochondria, as a minor or a labile species which would have escaped detection. However, it should be pointed out that in soybean, only one mt tRNA^{Trp} has been detected after aminoacylation of total mt tRNAs with ^{3}H or ^{14}C-tryptophan using mitochondrial, chloroplast or E.coli enzymes and fractionation on RPC-5 column (13).

As in spinach cp tRNA^{Trp} (10) in both bean mt and cp tRNAs^{Trp} there is a U_{11} : A_{24} base pair, which is also found in a tRNA^{Trp} of a Su$^+$ UGA strain of E.coli capable of decoding UGA (23, 24). This suppressor tRNA^{Trp} has a CCA anticodon (as the wild type E.coli tRNA^{Trp}), but its U_{11} : A_{24} base-pair is considered as being responsible for its suppressor activity (the wild type E.coli tRNA^{Trp} has a U_{11} : G_{24} base-pair). It would be interesting to see whether bean mt and cp tRNAs^{Trp}, which have a U_{11} : A_{24} base-pair, are able to read UGA in a cell-free protein synthesizing system.

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