Structure of bean mitochondrial tRNA^{Phe} and localization of the tRNA^{Phe} gene on the mitochondrial genomes of maize and wheat

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Bean mitochondrial tRNA^{Phe}, purified by RPC-5 chromatography and two-dimensional gel electrophoresis, has been sequenced using in vitro post-labeling techniques. It is the first plant mitochondrial tRNA sequenced. It shows 76% homology with bean chloroplast tRNA^{Phe} and has many features characteristic of prokaryotic tRNAs^{Phe}. It was used as a probe to localize the tRNA^{Phe} gene on the mitochondrial genomes of maize and wheat.

Plant mitochondria *Mitochondrial tRNA* *tRNA^{Phe} structure* *Gene localization* *Evolution*

1. INTRODUCTION

Plant mitochondria contain their own DNA and a complete apparatus for protein synthesis, including tRNAs and aminoacyl-tRNA synthetases. In plants, only mitochondrial methionine tRNA genes (with a CAU anticodon) have been sequenced so far: one in wheat [1], two in maize [2], and one in *Oenothera* [3]. But the determination of the sequence of a mitochondrial tRNA gene does not show whether this gene is actually transcribed into a functional tRNA. Furthermore, the sequence of the anticodon is not sufficient to identify the tRNA, because the mitochondrial genetic code, which has been shown to differ from the universal code in mammals and fungi [4,5], may also differ in plants [6,7] and because it has been reported that a post-transcriptional modification can change the specificity of the anticodon: in spinach chloroplasts for instance, the gene for an isoleucine tRNA has a methionine anticodon [8]. It is therefore essential to check the mitochondrial nature of each purified tRNA by hybridization with mitochondrial DNA and to identify the tRNA by aminoacylation, before determining its sequence.

We report here the first plant mitochondrial tRNA sequence. Bean mitochondrial tRNA^{Phe} was selected because of the large background of sequence information available on tRNAs^{Phe} [9], and because the sequence of bean chloroplast tRNA^{Phe} had been previously determined in our laboratory [10].

Labeled bean mitochondrial tRNA^{Phe} was used as a probe in hybridization studies with cloned fragments of maize and wheat mitochondrial DNA to localize the tRNA^{Phe} gene on the mitochondrial genome maps which have been recently established for these two species [11,12].

2. MATERIALS AND METHODS

Total mitochondrial (mt) tRNA was prepared from dark-grown bean (*Phaseolus vulgaris*) hypocotyls, as described [13] and fractionated on an RPC-5 column [14] using an NaCl gradient

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from 0.5 to 1.1 M in 0.01 M Na acetate buffer, pH 4.7, containing 0.01 M $MgCl_2$. Mitochondrial $tRNA^{Phe}$ was identified by aminoacylation, using either *E. coli* or wheat germ aminoacyl-tRNA synthetases and [^{3}H]phenylalanine [15]. Fractions containing mt $tRNA^{Phe}$ were pooled, concentrated and further subjected to two-dimensional polyacrylamide gel electrophoresis [16]. One of the major spots among the 14 spots separated on the gel was found by aminoacylation to contain a $tRNA^{Phe}$. From 16 kg of dark-grown bean hypocotyls, 10 μg pure mt $tRNA^{Phe}$ were obtained.

The nucleotide sequence of mt $tRNA^{Phe}$ was determined using in vitro post-labeling techniques [17] and approaches previously described [18].

For hybridization studies, mt $tRNA^{Phe}$ was enzymatically labeled at the 3′-end as in [19] with the following modifications: mt $tRNA^{Phe}$ (0.1–0.2 μg) was first denatured for 1 min at 100°C in 25% dimethyl sulfoxide (v/v) and incubated at 15°C for 3 h in 5 μl of a reaction mixture containing 3–6 μM [5′-^{32}P]pCp (3000 Ci/mmol), 50 μM ATP, 20 mM Tris-HCl (pH 7.5), 2 mM dithiothreitol, 15 mM $MgCl_2$, 15% dimethyl sulfoxide (v/v) and 0.25 unit T4 RNA ligase per μg tRNA. The labeled tRNA was separated from unreacted nucleotides by chromatography on a 0.1 ml RPC-5 column. The tRNA was eluted from the column with 6 × SSC and used directly for hybridization studies. A specific activity of 30 × 10^6 Cerenkov cpm per μg tRNA was currently obtained.

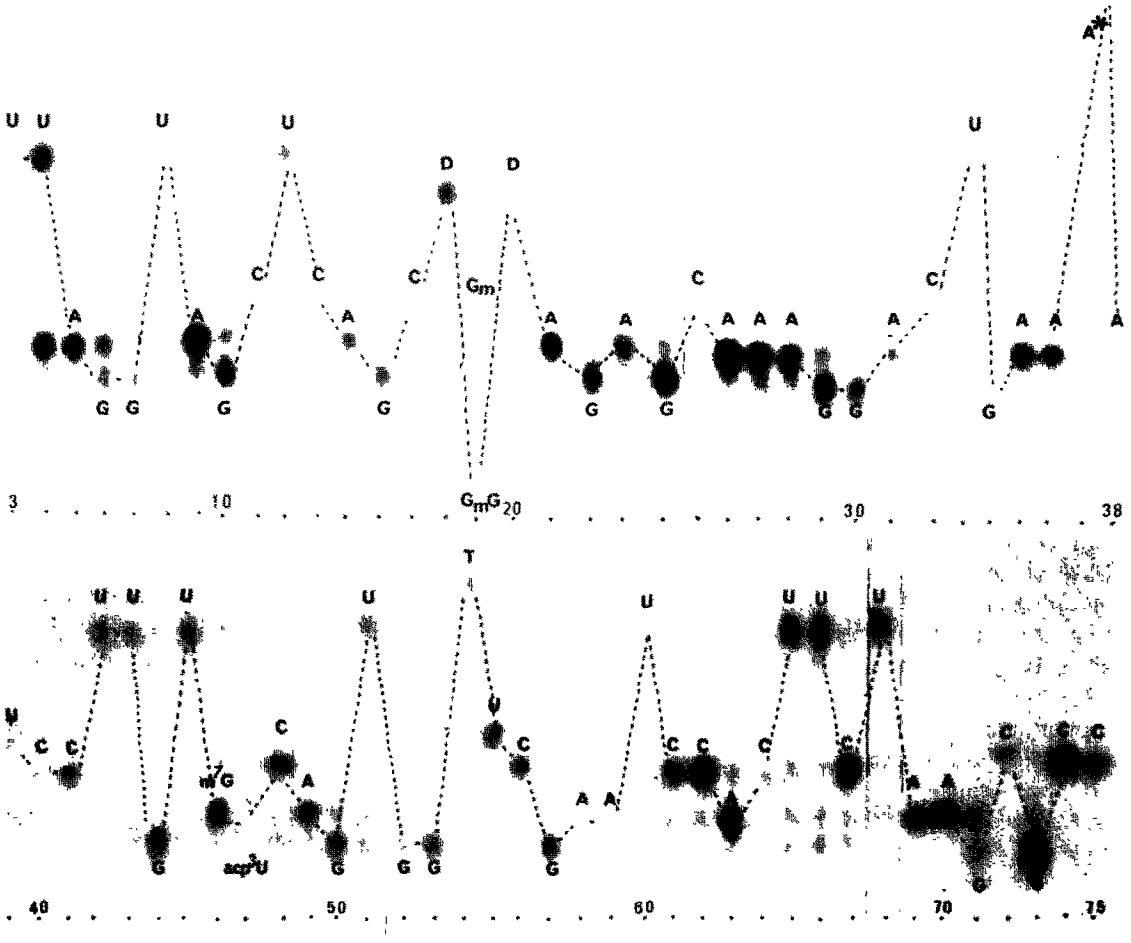

Fig.1. Sequence analysis of bean mitochondrial $tRNA^{Phe}$ using the Stanley and Vassilenko technique.

For the localization of its gene on the maize mitochondrial genome, the labeled $tRNA^{Phe}$ was hybridized to a set of cosmid clones of maize mitochondrial DNA digested with *Sma*I or *Xho*I and blotted on Gene Screen Plus (New England Nuclear). These blots were obtained from D. Lonsdale, Plant Breeding Institute, Cambridge, England [11].

For the localization of its gene on the wheat mitochondrial genome, the labeled $tRNA^{Phe}$ was hybridized to a dot blot of the 53 *Sal*I cloned fragments of wheat mitochondrial DNA covering the whole genome. The clones were obtained from F. Quétier and B. Lejeune, Orsay, France [12].

Filters were hybridized with the radioactive probe at 65°C for 20 h in 1 M NaCl, 1% SDS, 50 mM Tris-HCl, pH 8.0, 1 mM EDTA. Filters were washed twice in hybridization buffer at 65°C and twice in 0.1 × SSC at room temperature. Autoradiography using Kodak XAR-5 film was done at −80°C using intensifier screens.

3. RESULTS

The data obtained with the technique of Stanley and Vassilenko [20] gave the sequence of mt $tRNA^{Phe}$ from position 3 to 73 (fig.1). Residues at positions 1 and 2 were determined by analysis of the 5′-end oligonucleotides obtained by action of pancreatic and T1 RNases on (5′-^{32}P)-labeled mt $tRNA^{Phe}$. Further data, obtained from read-off sequencing gels and from mobility-shift analyses, confirmed these results. The structure of bean mt $tRNA^{Phe}$ is shown in fig.2.

Hybridization of the 3′-end-labeled bean mt $tRNA^{Phe}$ to the complete set of cosmid clones of maize mitochondrial DNA (fertile cytoplasm) allowed us to localize the $tRNA^{Phe}$ gene on an 8.1 kb *Sma*I fragment contained in the cosmid 9-1E5 [11] as shown in fig.3A. There is only one *Sma*I hybridizing fragment suggesting that there is only one $tRNA^{Phe}$ gene in the maize mitochondrial genome (unless there are several adjacent $tRNA^{Phe}$ genes on the same fragment). The $tRNA^{Phe}$ gene is located at about 10 kb from the S2 sequence involved in maize male sterility (fig.3A).

No hybridization of bean mt $tRNA^{Phe}$ was obtained with maize chloroplast DNA digested by *Sma*I, in spite of the high degree of homology observed between bean mt $tRNA^{Phe}$ and

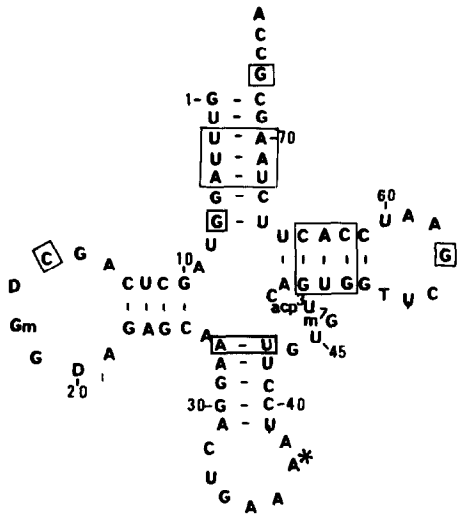

Fig.2. Nucleotide sequence of bean mitochondrial $tRNA^{Phe}$. Boxes indicate the parts of the molecule where the bean mt $tRNA^{Phe}$ differs from bean chloroplast $tRNA^{Phe}$. A*(37) = i^6A or ms^2i^6A.

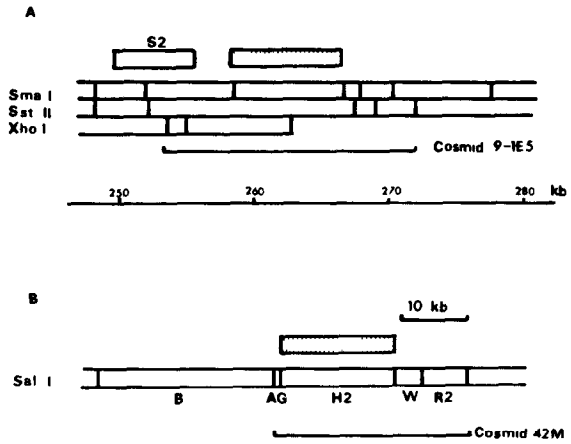

Fig.3. Localization of the $tRNA^{Phe}$ gene on the mitochondrial genomes of maize and wheat. (A) Partial map of the maize mt genome showing the 8.1 kb *Sma*I fragment of cosmid 9-1E5 containing the $tRNA^{Phe}$ gene. Ordinates are from [11]. The S2 box indicates the location of the S2 sequence involved in maize male sterility [11]. (B) Partial map of the wheat mt genome showing the H_2 (16.8 kb) *Sal*I fragment of cosmid 42 M [12] containing the $tRNA^{Phe}$ gene. The dotted boxes show the fragments containing the $tRNA^{Phe}$ gene.

Table 1

Percentage of homology between bean mitochondrial $tRNA^{Phe}$ and various sequenced $tRNAs^{Phe}$ or $tRNA^{Phe}$ genes*

Species	Percentage
Bacillus stearothermophilus	80.2
Bacillus subtilis	77.6
Euglena gracilis chloroplast	77.6
Spinach chloroplast	77.6
Bean chloroplast	76.3
Agmenellum quadruplicatum	76.3
Blue green algae – *Cyanobacterium* sp.	76.3
Rhodospirillum rubrum	73.6
Escherichia coli	71.0
Tetrahymena pyriformis mitochondria	65.8
Mycoplasma sp.	64.5
Mammals	63.1
Bombyx mori	63.1
Bovine lens	61.8
Euglena gracilis cytoplasm	61.8
Lupin	61.8
Drosophila melanogaster	60.5
Yeast mitochondria	60.5
Wheat germ, pea, barley, rape	59.2
Halobacterium volcanii	59.2
Yeast cytoplasm	56.5
Human mitochondria	56.5
Neurospora crassa	55.2
Saccharomyces pombe	55.2
Aspergillus nidulans mitochondria*	55.2
Mouse mitochondria*	53.9
Rat mitochondria*	51.3
Bovine mitochondria*	51.3

All these tRNA and tRNA gene sequences can be found in [9], except that of *Tetrahymena pyriformis* mt $tRNA^{Phe}$ [22] and *Halobacterium volcanii* $tRNA^{Phe}$ [25]

chloroplast $tRNAs^{Phe}$ from various sources (approx. 77%, see table 1).

Hybridization of the labeled $tRNA^{Phe}$ with the 53 cloned *Sal*I fragments contained in the wheat mitochondrial genome [12] allowed us to localize the $tRNA^{Phe}$ gene on fragment H2 (16.8 kb) as shown in fig.3B. As in maize, the mt $tRNA^{Phe}$ gene appears to be present only once in the wheat mitochondrial genome.

4. DISCUSSION

Bean mt $tRNA^{Phe}$ is 76 nucleotides long, if one includes the CCA terminus which is not encoded by the plant mitochondrial genome [1–3]. The reported sequence contains the characteristic invariant and semi-invariant nucleotides of the usual cloverleaf structure. It contains the nucleotides common to all sequenced $tRNAs^{Phe}$, with the exception of position 73 where a G is found instead of an A in all other $tRNAs^{Phe}$. The GAA anticodon can recognize both UUU and UUC codons which specify phenylalanine according to the universal code.

Bean mt $tRNA^{Phe}$ does not display the unusual features reported for yeast [21] and *Tetrahymena* [22] mt $tRNAs^{Phe}$, namely, the lack of T in the ψC loop observed in both yeast and *Tetrahymena*, an extranucleotide in the base-paired region of the ψC loop found in yeast and an extra 5′-end nucleotide found in *Tetrahymena*.

It is interesting to note the high degree of sequence homology between bean mt $tRNA^{Phe}$ on the one hand and prokaryotic and chloroplast $tRNAs^{Phe}$ on the other (table 1). Boxes in fig.2 indicate the parts of the molecule where the bean mt $tRNA^{Phe}$ differs from its bean chloroplast counterpart. Of the 18 nucleotides which are different, 6 (3 base-pairs) are located in the amino acid stem and 6 (3 base-pairs) in the Tψ stem. Furthermore, minor bases found at position 37 (i^6A or ms^2i^6A) and at position 47 (acp^3U) have already been found in a few chloroplast tRNAs and in a number of *E. coli* tRNAs [9]. In fact, wheat, maize and *Oenothera* mt $tRNA^{Met}$ gene sequences also display high homology with prokaryotic and chloroplast $tRNA^{Met}$ [1–3]. These results suggest that plant mitochondrial and chloroplast tRNA genes derive from common ancestor genes, which is in agreement with the hypothesis of the endosymbiotic origin of chloroplasts and mitochondria.

Hybridization of bean mt $tRNA^{Phe}$ to cloned mitochondrial DNA of maize and wheat has allowed the localization of the corresponding gene on the maps of maize and wheat mitochondrial DNA which have been recently established [11,12]. These heterologous hybridization experiments suggest that the mt $tRNA^{Phe}$ gene sequences are highly conserved in higher plant mitochondrial genomes, which is also the case in the various chloroplast genomes [23].

There seems to be only one $tRNA^{Phe}$ gene (unless there are several adjacent $tRNA^{Phe}$ genes on the

same fragment) in the maize and wheat mitochondrial genome. This is also true in the mitochondrial genome of carrot (V. Gruber, G. Belliard and J.M. Grienenberger, unpublished). The $tRNA^{Phe}$ sequence therefore does not seem to be involved in the mitochondrial recombination events which are characteristic of plant mitochondrial genetics, in contrast for instance to the mitochondrial rRNA genes in wheat [24].

As the mt $tRNA^{Phe}$ gene is located in maize close to the S2 sequence, which is integrated in the mitochondrial genome in the fertile strains (fig.3A), but exists as a free plasmid-like molecule in the S male-sterile strains, it would be interesting to study the localization of the mt $tRNA^{Phe}$ gene in male-sterile mitochondria.

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