Localization, sequence and expression of the gene coding for tRNA^Pro (UGG) in plant mitochondria

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Abstract

The four Sal I fragments of wheat mitochondrial DNA containing the 18S and 5S ribosomal RNA genes were screened for the presence of tRNA genes. Upon sequencing, a tRNA^Pro (UGG) gene was found in two of these four fragments. The localization of the corresponding gene on the maize mitochondrial genome was established. Transcriptional studies have shown that this gene is transcribed in wheat and maize mitochondria. The sequence of the corresponding tRNA^Pro (UGG) of bean mitochondria was determined using in vitro post-labeling techniques.

Introduction

The plant mitochondrial (mt) genome has been shown to consist of a number of circular molecules, deriving from a master chromosome by recombination events, plus a set of small plasmid-like molecules [1, 2]. Together, these DNA molecules constitute large genomes, the size of which differ depending on the plant considered (200–2500 kb) [1, 3, 4], much more complex than animal or fungal mt genomes [5, 6].

Only a few plant mt tRNA genes and their flanking regions have been sequenced so far [7–9]. Due to the high homology between these genes and their chloroplast (cp) counterparts (up to 98%), it was postulated that the plant mt and cp tRNA genes have evolved from common ancestor genes [10]. In addition, it has been shown that the plant mt genome contains cp DNA insertions sometimes harbouring cp tRNA genes [11]. For instance, the maize mt genome contains a 12 kb cp DNA insertion [12] which carries cp tRNA genes coding for tRNA^Val, tRNA^His and the 3' half of tRNA^Ala. The distinction between unexpressed pseudogenes present in cp DNA insertions and functional plant mt tRNA genes is therefore not evident.

In wheat mitochondria, a sequence harbouring the 5S and 18S rRNA genes and a tRNA^Met gene has been shown to be repeated four times in the genome. The organization of the flanking regions of this repeated sequence suggests that a recombination event is responsible for the presence of these four fragments [13].

We report the localization and the sequence of a tRNA^Pro (UGG) gene which is present in two of the four clones containing this repeated fragment. We also provide evidence that this tRNA^Pro (UGG) gene is expressed, in contrast to another, partial or complete, tRNA^Pro (UGG) gene present as a cp insertion in the mt genome of wheat and maize,
respectively [14]. That the tRNA\textsuperscript{Pro} (UGG) gene mapped and sequenced in this study is transcribed was confirmed by the fact that its sequence is 100% homologous to that of bean mt tRNA\textsuperscript{Pro} (UGG) which was also determined in this work.

**Material and methods**

*Isolation of wheat mt tRNAs*

Wheat mt tRNAs were extracted from mitochondria purified by sucrose gradient centrifugation [15]. The mitochondria were lysed with 1% Triton X100 and the membranes were pelleted. The tRNAs were purified from the supernatant by phenol extraction, 1 M NaCl precipitation of the high molecular weight RNAs, DEAE-cellulose chromatography of the supernatant and ethanol precipitation.

*Localization and sequencing of the wheat tRNA\textsuperscript{Pro} gene*

For the localization of the tRNA genes in the wheat mt genome, the total wheat tRNAs were specifically labeled at their 3' end, using α-[\textsuperscript{32}P]-ATP, in the presence of yeast tRNA nucleotidyl-transferase, after limited digestion of the terminal CCA sequence with snake venom phosphodiesterase [16]. The labeled tRNAs were hybridized as already described [17] to a dot blot of the 53 Sal I cloned fragments of wheat mt DNA covering the whole genome [18]. These clones were obtained from F. Quetier and B. Lejeune, Orsay (France). The tRNA\textsuperscript{Pro} gene was located in a 750 bp Hind III fragment of the F2 Sal I clone. This fragment was cloned in M13mp10 and sequenced by the dideoxynucleotide chain termination method [19].

*Localization of the maize mitochondrial tRNA\textsuperscript{Pro} gene*

The mt tRNA\textsuperscript{Pro} gene was localized on the maize mt genome using an oligodeoxynucleotide (26 nucleotides long) complementary to the 3' end of the wheat mt tRNA\textsuperscript{Pro} gene. This oligodeoxynucleotide was labeled with γ-\textsuperscript{(32P)}-ATP as previously described [14] and hybridized to a set of maize mt cosmid clones digested with Sma I or Xho I and blotted on GeneScreen Plus. These blots were obtained from D. Lonsdale (PBI, Cambridge, UK). The hybridization and subsequent washings were carried out as already described [14], except that the washing temperature was raised to 70°C in relation to the Td of the oligodeoxynucleotide probe which was used [20].

*Characterization of the transcripts of the tRNA\textsuperscript{Pro} genes*

Wheat and maize mt and cp total RNAs were isolated as previously described [21]. The RNAs were separated on a denaturing formaldehyde agarose gel and blotted on GeneScreen Plus. These "Northern" blots were hybridized with labeled oligodeoxynucleotides complementary to the mt or cp tRNA\textsuperscript{Pro} genes.

*Isolation and purification of bean mt tRNA\textsuperscript{Pro}*

Total bean mt tRNAs were prepared from dark-grown bean (Phaseolus vulgaris) hypocotyls as previously described [17] and fractionated on a RPC-5 column [10]. Using E. coli aminoacyl-tRNA synthetases, tRNA\textsuperscript{Pro} were identified by aminoacylation; fractions containing the tRNA\textsuperscript{Pro} were pooled, concentrated and subjected to two-dimensional polyacrylamide gel electrophoresis. The spot containing tRNA\textsuperscript{Pro} was identified and the pure tRNA was eluted. From 3.2 mg of total bean mt tRNAs, 1.8 μg of pure tRNA\textsuperscript{Pro} was purified.

*Sequencing of bean mt tRNA\textsuperscript{Pro}*

One μg of pure tRNA\textsuperscript{Pro} was partially hydrolysed in deionized formamide at 80°C for 3 min. After