Plant nuclear tRNA^{Met} genes are ubiquitously interrupted by introns

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Abstract

We have isolated three independent clones for nuclear elongator tRNA^{Met} genes from an Arabidopsis DNA library using a tRNA^{Met}-specific probe generated by PCR. Each of the coding sequences for tRNA^{Met} in these clones is identical and is interrupted by an identical 11 bp long intervening sequence at the same position in the anticodon loop of the tRNA. Their sequences differ at two positions from the intron in a soybean counterpart. Southern analysis of Arabidopsis DNA demonstrates that a gene family coding for tRNA^{Met} is dispersed at at least eight loci in the genome. The unspliced precursor tRNA^{Met} intermediate was detected by RNA analysis using an oligonucleotide probe complementary to the putative intron sequence. In order to know whether introns commonly interrupt plant tRNA^{Met} genes, their coding sequences were PCR-amplified from the DNAs of eight phylogenetically separate plant species. All 53 sequences determined contain 10 to 13 bp long intervening sequences, always positioned one base downstream from the anticodon. They can all be potentially folded into the secondary structure characteristic for plant intron-containing precursor tRNAs. Surprisingly, GC residues are always present at the 5'-distal end of each intron.

Introduction

Some eukaryotic nuclear tRNA genes contain intervening sequences, which must be accurately removed from precursor tRNAs (pre-tRNAs) to give functional tRNAs. These intron sequences, ranging from 14 to 60 bp in yeast, for instance, share no homology among different tRNA gene families [28]. Instead, a characteristic secondary structure of intron-containing pre-tRNAs can generally be predicted from their nucleotide sequences [14] and/or by limited ribonuclease digestion [11], i.e. base-pairing between several bases in the anticodon loop and the intron allows the extension of the anticodon stem in the conventional tRNA structure. This consensus structure predicted in all pre-tRNAs is necessary for precise excision of the intron by the splicing endonuclease, according to several lines of evidence obtained from in vitro tRNA splicing studies [13, 15, 29].

In plants, nuclear genes for tRNA^{Tyr} from tobacco [9, 19, 26], Arabidopsis [2, 20] and wheat [22] carry introns. Secondary structure deduced from the primary sequences and studies on in vitro splicing of pre-tRNAs transcribed from mutated versions of these genes [23] demonstrate that the intron-containing pre-tRNAs possess an extended anticodon stem as in other organisms and are recognized by a plant endonuclease which can excise introns faithfully. On the other hand, comparison of the specificities of nuclear extracts from yeast, HeLa cell and wheat germ for in vitro pre-tRNA splicing has revealed interesting differences [20]: introns in yeast or human pre-tRNAs cannot be removed in a plant extract, whereas those of plant pre-tRNAs can be removed in heterologous extracts. As for the definitive secondary structure predicted from a number of tobacco tRNA^{Tyr} genes, in spite of the variable size and heterogeneous sequence of the introns, 5' and
3' splice sites are always separated by 4 bp of an intron-anticodon secondary structure [9, 19, 26]. Taken together with the fact that this distance is 5 or 6 bp in yeast and vertebrate pre-tRNAs, one can speculate that the fixed arrangement of these sites in the tertiary structure of plant pre-tRNAs is specifically recognized by the plant enzyme [20]. Nevertheless, it is still not clear that substrate recognition of the plant endonuclease relies only on this feature of the pre-tRNA structure.

A soybean gene coding for elongator tRNA<sub>Met</sub>, interrupted by a 11 bp long intron, has been isolated [27]. The gene is predicted to display an identical secondary structure to some plant tRNA<sub>Tyr</sub> genes, potentially being a good substrate for splicing (Fig. 3A). Intron-containing genes for tRNA<sub>Tyr</sub> have been reported in plants as well as in Trypanosoma [17], yeast [14], human [12, 26] and Drosophila [21]; however, because the soybean elongator tRNA<sub>Met</sub> gene is the only example in eukaryotes, it is unclear whether all nuclear plant genes encoding tRNA<sub>Met</sub> carry intervening sequences that allow formation of the secondary structure common to the plant pre-tRNAs reported. In this study, we first attempted to isolate clones containing nuclear tRNA<sub>Met</sub> genes from an Arabidopsis DNA library, using a specific probe for tRNA<sub>Met</sub> generated by PCR. Then, we cloned the corresponding gene sequences from a variety of plant species by PCR; we expected that this would allow an extensive comparison among the plant pre-tRNAs reported. In this study, we show that all the tRNA<sub>Met</sub> genes cloned carry introns, whose sequences could contribute to the typical plant pre-tRNA structure. Furthermore, a pair of GC residues is conserved at the 5'-distal portion of every intron, implying an important role for pre-tRNA processing.

**Materials and methods**

**Plant materials**

Eight different plant species were used in this study: Arabidopsis thaliana (ecotype Landsberg erecta), pea (Pisum sativum L.), tobacco (Nicotiana tabacum cv. Petit Havana, line SRI), rice (Oryza sativa L.), ginkgo (Ginkgo biloba L.), fern (Rumohra standishii Ching), moss (Polytrichum juniperinum) and liverwort (Marchantia paleacea var. diptera).

**Preparation of plant DNAs and Arabidopsis tRNAs**

Total DNAs were isolated from whole plants of Arabidopsis, fern or moss, from leaves of tobacco or ginkgo and from embryonic axes of pea, essentially as described [8]. Rice DNA was prepared by extraction with phenol/chloroform from the phage lysate (2×10<sup>7</sup> pfu) of a λ GEM12 rice genomic library constructed by Dr A. Kato (Hokkaido University, Sapporo, Japan) and liverwort total DNA was provided by Dr S. Takio (Hiroshima University, Hiroshima, Japan). Unfractionated tRNAs were extracted from two-week-old seedlings of Arabidopsis by a slightly modified method using acid guanidinium thiocyanate/phenol/chloroform [7].

**Preparation of oligodeoxyribonucleotides**

The two oligonucleotides as PCR primers, designated MET<sup>5'</sup> (5'-GGGGTGTTGGCCGATTTGG-3': nucleotides (nt) 1-19) and MET<sup>3'</sup> (5'-TGGGGTGAGAGAGGCTC-3': nt 57-73) corresponding to the 5'-distal and complementary to the 3'-distal region of a soybean nuclear tRNA<sub>Met</sub> gene [27], shown in Fig. 3A, were synthesized with a Cyclone Plus DNA Synthesizer (Millipore). As a control for the PCR, two pairs of PCR primers (5'-TGCGGTGAGCGTGGATC/GGAACATC-3' and 5'-GCGGGGATAGCTCAGTTGG-3') specific for the 5' and 3' ends of Arabidopsis nuclear gene sequences for tRNA<sub>Phe</sub> [1] or tRNA<sub>Tyr</sub> [20], respectively, were synthesized in the same way. For northern blot analysis to detect the precursor tRNA, an oligonucleotide (METInt; 5'-CAGGATCACTCAGTAGC-3') complementary to a sequence region (nt 139-155 in Fig. 2) including the intron of the Arabidopsis tRNA<sub>Met</sub> gene was also prepared.

**PCR amplification of nuclear tRNA<sub>Met</sub> genes in plants**

Plant DNAs (0.1–1.0 μg) were used to amplify tRNA<sub>Met</sub> genes by PCR with 1 μM of each relevant primer, 250 μM dNTP and 1 unit of Taq DNA polymerase (Toyobo) using the GeneAmp 9600 PCR system (Perkin-Elmer). The PCR conditions were as follows: denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s and extension at 72 °C for 30 s, repeated 30 times. Aliquots of PCR products were run on a