Homology between Chloroplast and Prokaryotic Initiator tRNA

NUCLEOTIDE SEQUENCE OF SPINACH CHLOROPLAST METHIONINE INITIATOR tRNA*

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The nucleotide sequence of a chloroplast methionine initiator tRNA from spinach has been determined. Although from a euukaryotic organism, this tRNA strongly resembles prokaryotic initiator tRNAs. Spinach chloroplast tRNA\textsuperscript{Met} has a much higher sequence homology with prokaryotic initiator tRNAs (81 to 84\%) than with euukaryotic initiator tRNAs (64 to 69\%). In addition, it possesses the two unique features of prokaryotic initiator tRNAs, lacking a base pair between the 5'-terminal residue and the sixth nucleotide from the 3'-end and containing a T-U-C-A sequence in loop IV. Also, like prokaryotic initiator tRNAs, the chloroplast tRNA\textsuperscript{Met} is 77 nucleotides long and has few modified nucleosides (2'-O-methylguanosine, dihydrouridine, 7-methylguanosine, ribothymidine, and pseudouridine). This chloroplast initiator tRNA is strikingly different in sequence homology (55 to 62\%), number of residues, and structure from mitochondrial initiator tRNAs. Restriction enzyme mapping techniques have shown that the chloroplast tRNA\textsuperscript{Met} hybridizes to spinach chloroplast DNA.

A set of characteristic chloroplast tRNA features seems to be emerging from a comparison of this tRNA\textsuperscript{Met} and several other chloroplast tRNAs which have been completely or partially sequenced. All have a 2'-O-methylated G-G sequence in the dihydrouridine loop, and the sequence T-W-C-A, as opposed to T-U-C-G, is predominantly found in loop IV. This is the reverse of the situation encountered in the overall non-chloroplastic tRNA population.

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Substantial and well defined differences occur in the initiation processes of protein synthesis in prokaryotes and euukaryotic cytoplasm. In particular, there are specific and characteristic differences between initiator methionine tRNAs from prokaryotic and euukaryotic sources (1–7). Recently, two mitochondrial initiator tRNA sequences were determined and marked differences between them and all other initiator tRNAs were observed (8, 9). Since the structures of initiator tRNAs are so indicative of their prokaryotic or euukaryotic origins, a comparison of initiator tRNA sequences from both mitochondria and chloroplasts with those from prokaryotic and euukaryotic cells may aid in the evaluation of the possible functional and/or evolutionary relationships of these organelles to prokaryotes, euukaryotes, or to each other. We have therefore determined the sequence of a chloroplast initiator methionine tRNA.

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The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; A\textsubscript{260} units, one absorbance (A\textsubscript{260}) unit is defined as that amount of material/ml of solution which produces an absorbance of 1 in a 1 cm light path at a wavelength of 260 nm; NaOAc, sodium acetate; PEL, polyethyleneimine.

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Stanley and Vassilenko (19) and “mobility shift” analysis (10, 20, 21). RNA sequence gels, using partial enzymatic reaction conditions previously described (10, 11), were run on both 3' and 5'-end labeled spinach chloroplast tRNA\(^{\text{Met}}\). A short electrophoretic run on a 20% polyacrylamide gel of partial digestion products of [\(5'-'^{32}\text{P}\)]tRNA\(^{\text{Met}}\) gave the sequence of residues 4 to 39 as G-G-G-G-U-A-G-A-C-A-G-U-U-X-G-(D)-A-G-X-U-X-G-Y-A-A-G-G-X-U-C-A-A-A. A longer electrophoretic run of partial digestion products of [\(5'-'^{32}\text{P}\)]tRNA\(^{\text{Met}}\) gave the sequence of residues 17 to 54 as U-U-X-G-(D)-A-G-X-U-C-G-Y-A-A-G-G-X-U-C-A-A-X-U-U-X-G-A-G-X-U-C-A-X-G-G-G. A long electrophoretic run of partial digestion products of [\(3'-'^{32}\text{P}\)]tRNA\(^{\text{Met}}\) gave the sequence of residues 9 to 60 as A-G-A-G-C-A-G-U-U-X-G-Y-A-A-G-G-X-U-G-C-A-A-U-A-A-A-C-A-C-G-G-U-G-Y-A-Y-G-G-X-X-(A)-A-A-A. A shorter electrophoretic run of the partial digestion products of [\(3'-'^{32}\text{P}\)]tRNA\(^{\text{Met}}\) gave the sequence of residues 50 to 76 as A-G-G-G-X-X-C-A-A-A-U-X-X-U-G-U-U-X-U-X-G-C-A-A-A-C-C.

In addition to RNA sequence gels, the procedures of Stanley and Vassilenko (19) and Gupta and Randerath (22), involving analysis of 5',32P-labeled partial formamide-derived fragments, established the identity of all residues in this tRNA except residues 1, 74 to 77, and the Gm at residue 19. Fig. 2 shows the analysis of residues 43 to 71 using these procedures. That position 19 is the locus of the Gm was shown by the fact that the product of a complete ribonuclease T\(\text{I}\) digestion of a formamide fragment corresponding to residue 19 had a mobility identical to that of 5',32P-labeled spinach chloroplast tRNA\(^{\text{Met}}\) in these solvent systems. This and other [\(5'-'^{32}\text{P}\)]formamide-derived oligonucleotides which had modified nucleotides at their 5'-termini were also digested with nuclease P\(_1\), and the resulting [\(5'-'^{32}\text{P}\)]nucleoside 5'-monophosphates were fractionated by two-dimensional thin layer chromatography (TLC) on cellulose plates (18). Fig. 2C shows the autoradiograms of three such analyses.

In addition to RNA sequence gels and formamide fragment analysis, “mobility-shifts” (10, 20, 21) were run on either 5'- or 3'-labeled tRNA\(^{\text{Met}}\). These analyses aided in the determination of residues 1 to 9, 36 to 46, and 64 to 76.

**Hybridization of Spinach Chloroplast tRNA\(^{\text{Met}}\)—**The [\(5'-'^{32}\text{P}\)]tRNA\(^{\text{Met}}\) was hybridized to restriction fragments of spinach chloroplast DNA which were generated by the enzymes Sal I and Pst I, using methods previously described (11, 23–25). Spinach chloroplast [\(5'-'^{32}\text{P}\)]tRNA\(^{\text{Met}}\) hybridized to the same Sal I and Pst I fragments that have been shown to contain the genes for two spinach chloroplast methionine tRNAs, Met\(\text{i}\) and Met\(\text{c}\) (26). Therefore, the spinach chloroplast tRNA\(^{\text{Met}}\) must be encoded on the spinach chloroplast genome.

**DISCUSSION**

Initiator methionine tRNAs from prokaryotic and eukaryotic cytoplasm possess specific structural features which clearly distinguish them from each other and from most non-initiator tRNAs (1–7). All prokaryotic initiator tRNA\(_\text{s}\) sequenced to date lack a base pair between the 5'-terminal nucleotide and the fifth nucleotide from the 3'-end, and contain a T(or U)-ψ-C-A(or G) sequence in loop IV, while eukaryotic initiator tRNAs contain a standard base pair A\(_\text{i}\);U\(_\text{i}\) and A\(_\text{i}\);G\(_\text{i}\) (Przybylo et al., 1972).

The numbering system used here for tRNA nucleotide positions is the standard system of Ref. 1 (Sprinzl et al., compilation of tRNA sequences).
With the determination of the sequence of this chloroplast tRNA^{Met} and its comparison with other chloroplast tRNAs (11, 27-30), and spinach chloroplast elongator methionine tRNA^{3} and two other partial sequences of spinach chloroplast tRNAs (3), several features characteristic of chloroplast tRNAs appear to be emerging. These include a methylated G-G sequence in the dihydrouridine loop and a predominance of T-Ψ-C-A as compared to T-Ψ-C-G in loop IV, which is the reverse of the relative abundances of these sequences observed in non-chloroplast tRNAs. As more tRNA sequences from chloroplasts are determined, it will be interesting to determine how general these “chloroplast-specific” tRNA characteristics are.

After this work was completed, the sequence of a bean (Phaseolus vulgaris) chloroplast tRNA^{Met} was determined (27). This tRNA is 92% homologous to the spinach chloroplast tRNA^{Met} reported here, and is in excellent agreement with the spinach chloroplast tRNA^{Met} in terms of its homology with prokaryotic (as opposed to eukaryotic) initiator tRNAs. Bean chloroplast tRNA^{Met} also conforms to all of the characteristic “chloroplast-specific” as well as “initiator-specific” tRNA features discussed above.

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**Fig. 3.** Nucleotide sequence of spinach chloroplast methionine initiator tRNA.
Nucleotide Sequence of Spinach Chloroplast tRNA\textsubscript{Met}

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