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The nucleotide sequence of spinach chloroplast methionine elongator tRNA

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ABSTRACT

The nucleotide sequence of spinach chloroplast methionine elongator tRNA (sp.chl. $tRNA^{Met}_m$) has been determined. This tRNA is considerably more homologous to E. coli $tRNA^{Met}_m$ (67% homology) than to the three known eukaryotic $tRNA^{Met}_m$ (50-55% homology). Sp.chl. $tRNA^{Met}_m$, like the eight other chloroplast tRNAs sequenced, contains a methylated GG sequence in the dihydrouridine loop and lacks unusual structural features which have been found in several mitochondrial tRNAs.

INTRODUCTION

Protein synthesis in mitochondria has substantial differences in comparison with that occuring in prokaryotes and eukaryotic cytoplasm. In particular, mitochondria contain tRNAs which possess unusual structural features (1-4) and the reading of the genetic code in mitochondria has specific and well defined differences from that which occurs in non-mitochondrial systems (5-10). The nature of protein synthesis in chloroplasts is not as well defined as in mitochondria. As part of a general study to obtain a better understanding of the nature of protein synthesis as it occurs in chloroplasts, we have determined the nucleotide sequence of spinach chloroplast methionine elongator tRNA.

MATERIALS AND METHODS

The isolation of chloroplasts from Spinacia oleracea L. var 424, the isolation of total spinach chloroplast tRNA and the methods used in nucleotide sequence determination and hybridization have been discussed previously (11-13).

RESULTS

Purification of tRNA. Crude spinach chloroplast tRNA, purified as previously described (13) was fractionated on two successive RPC-5 columns

as shown in Fig. 1. This procedure yielded highly purified methionine elongator tRNA (fractions 153 and 154 of Fig. 1B) which was suitable for nucleotide sequence determination. This tRNA was distinguished from spinach chloroplast (sp.chl.) $tRNA^{Met}_{f}$ by its inability to be formylated with crude E. coli transformylase (13).

Sequence analysis. The modified residues present in sp. chl. $tRNA^{Met}_{m}$ were determined by the methods of Randerath and Randerath (14) and Silberklang et al. (15). The nucleotide sequence of sp.chl. $tRNA^{Met}_{m}$ was determined using three different and partially overlapping sequencing procedures; formamide fragment analysis, RNA sequence gels and mobility shifts. The formamide fragment analysis method as developed by Stanley and Vassilenko

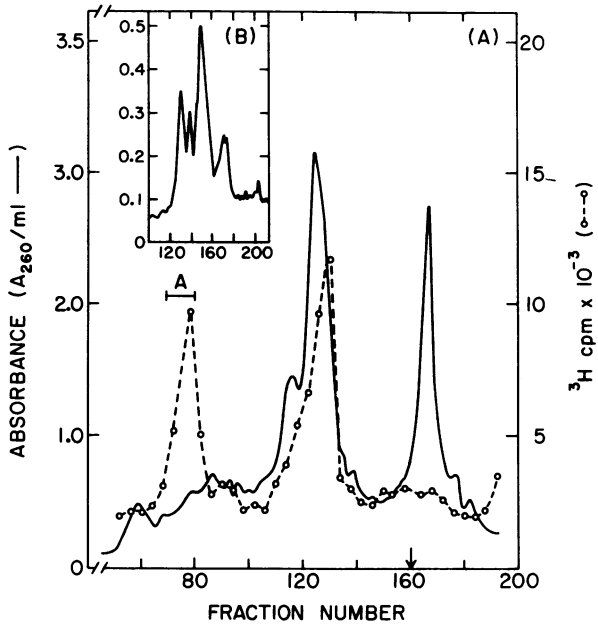

Fig. 1. Isolation of spinach chloroplast methionine elongator tRNA on RPC-5. A, total spinach chloroplast tRNA was chromotographed on an RPC-5 column (95 x 0.6cm) with a concave NaCl gradient generated from 500ml of 0.40M NaCl and 250 ml of 0.80M NaCl, both in Buffer A (10mM NaOAc, pH 4.5, 5mM $MgCl_2$). Fractions of 4ml were collected at a flow rate of 0.57 ml/min. At the end of the gradient (see arrow) additional 0.80M NaCl in Buffer A was applied. Open circles represent methionine acceptor activity. B, fractionation of pooled fraction A from Fig. 1A on an RPC-5 column (45 x 0.4cm) with a concave NaCl gradient generated from 200ml of 0.40M NaCl and 100ml of 0.80M NaCl, both in Buffer B (10mM Tris pH 7.6, 5mM $MgCl_2$, 1mM $Na_2S_2O_3$). Fractions of 1ml were collected at a flow rate of 0.2ml/min. Fractions 153 and 154 were used for the sequence determination and methionine acceptor assay.

(16) and modified by Gupta and Randerath (17) provided the most sequence information of the three methods employed and allowed an unambiguous assignment of residues 3 to 71. Part of this sequence, from residue A_{14} to residue G_{67} is shown in Fig. 2. The identification of GmG in the dihydrouridine loop, and all other modified nucleotides obtained during the formamide fragment analysis method were identified as described previously (12).

RNA sequence gels (11,18-20) were used to analyze both [5'-^{32}P] and [3'-^{32}P] sp.chl. $tRNA^{Met}_m$. The RNA sequence gel of [5'-^{32}P] sp. chl. $tRNA^{Met}_m$ confirmed the nucleotide assignment of residues 7 to 44 and 48 to 53. Two RNA sequence gels were run on [3'-^{32}P] sp.chl. $tRNA^{Met}_m$. The first gel (long electrophoretic run) confirmed the nucleotide assignments of residues 14 to 59. The second gel (short electrophoretic run) both confirmed residues 34 to 71 and assigned residues 72 and 73.

The 5' and 3' ends of sp.chl. $tRNA^{Met}_m$ were determined by mobility shift procedures (15). A mobility shift analysis of [5'-^{32}P] sp.chl. $tRNA^{Met}_m$ gave residues A_1 to U_{12} and a similar analysis of [3'-^{32}P] sp.chl. $tRNA^{Met}_m$ gave residues U_{65} to A_{76}. In addition, several mobility shifts were run to provide additional confirmation of various regions of the molecule including residues $G_{m}17$ to A_{23} and A_{35} to G_{44}.

These three different sequencing procedures, formamide fragment analysis, RNA sequence gels and mobility shifts, allowed us to unambiguously deter-

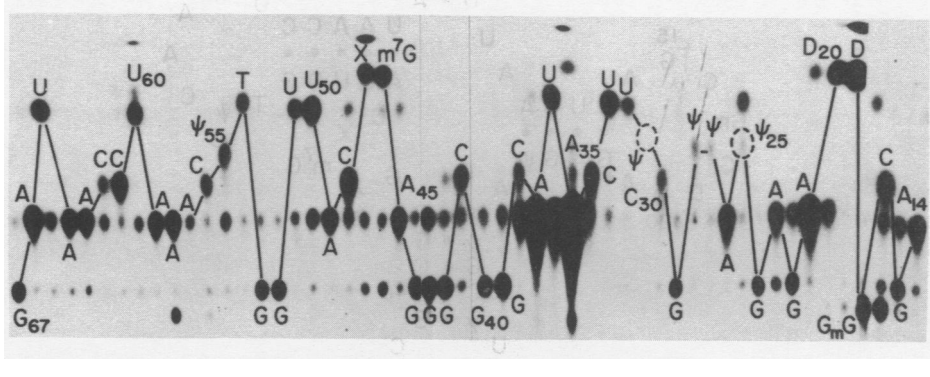

Fig. 2. Analyses of [5'-^{32}P]-labeled partial formamide fragments. One - dimensional PEI-cellulose TLC analysis of [5'-^{32}P]-labeled complete RNase T_2 digestion products of fragments produced by partial formamide hydrolysis of sp.chl. $tRNA^{Met}_m$ in the ammonium sulfate solvent system of Gupta and Randerath (17). Residues in dotted circles became apparent upon longer exposure. All modified residues were confirmed by two-dimension cellulose TLC analysis as previously described (12,13).

mine the complete nucleotide sequence of sp.chl. $tRNA^{Met}_{m}$ and provided much overlapping and confirmatory data.

Hybridization of spinach chloroplast $tRNA^{Met}_{m}$. The [5'-^{32}P] sp.chl. $tRNA^{Met}_{m}$ was hybridized to restriction fragments of sp.chl. DNA which were generated by the enzymes Sal I and Pst I respectively, using methods previously described (12,13,21-23). The [5'-^{32}P] sp.chl. $tRNA^{Met}_{m}$ hybridized to a 12.8 megadalton Sal I fragment and to a 7.6 megadalton Pst I fragment. These fragments are derived from a region of the spinach chloroplast genome to which sp.chl. $tRNA^{Met}_{2}$ has been mapped (24,25).

DISCUSSION

The nucleotide sequence of sp.chl. $tRNA^{Met}_{m}$ has been determined and is shown in Fig. 3. This tRNA, like E. coli $tRNA^{Met}_{m}$, which is the only prokaryotic $tRNA^{Met}_{m}$ of known sequence, contains 76 nucleotides. Eukaryotic $tRNA^{Met}_{m}$

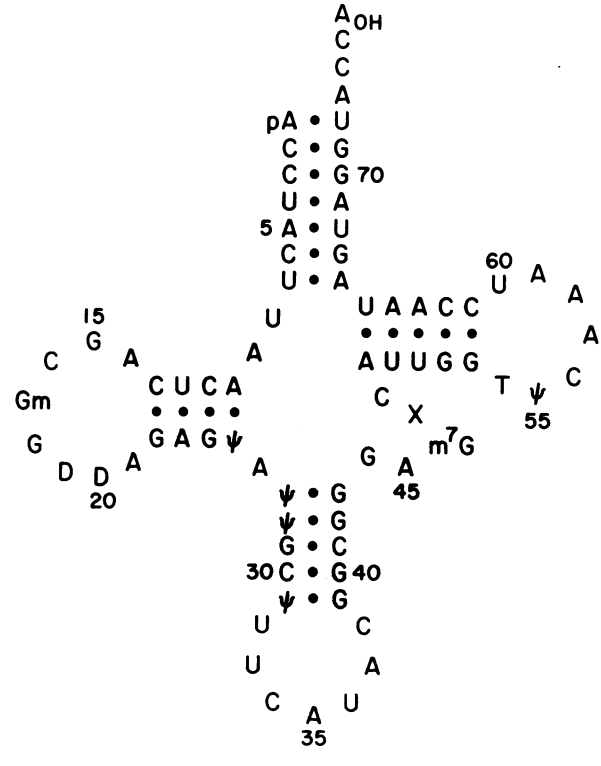

Fig. 3. Nucleotide sequence of spinach chloroplast methionine elongator tRNA.

(yeast, rabbit liver and mouse myeloma) all contain 75 nucleotides. Sp.chl. $tRNA^{Met}_{m}$ also shows considerably more homology with prokaryotic $tRNA^{Met}_{m}$ (67% homology) than with eukaryotic $tRNA^{Met}_{m}$ (50-55% homology). Nevertheless, this homology of sp.chl. $tRNA^{Met}_{m}$ with prokaryotic $tRNA^{Met}_{m}$ is considerably lower than the homology shown by the recently sequenced sp.chl. $tRNA^{Met}_{f}$ (13) with prokaryotic $tRNA^{Met}_{f}$ (81-84% homology). Moreover, sp.chl. $tRNA^{Met}_{m}$ shows relatively little homology (49% homology) to sp.chl. $tRNA^{Met}_{f}$, although both tRNAs have identical TΨC loops.

Sp.chl. $tRNA^{Met}_{m}$ is the ninth chloroplast tRNA sequenced, the other eight being Euglena $tRNA^{Phe}$ (26), bean $tRNA^{Phe}$ (27), bean $tRNA^{Met}_{f}$ (28), spinach $tRNA^{Met}_{f}$ (13), spinach $tRNA^{Thr}_{3}$ (12), and three bean leucine tRNAs (29). All contain a methylated GG sequence in the dihydrouridine loop and, except for sp.chl. $tRNA^{thr}_{3}$, all lack unusual structural features such as have been found in several mitochondrial tRNAs (1-4). All except sp.chl. $tRNA^{thr}_{3}$ show greater homology to their prokaryotic compared to their eukaryotic counterparts.

Sp.chl. $tRNA^{Met}_{m}$ is the fifth methionine elongator tRNA sequenced to date; the others are from E. coli (30), yeast (31,32), rabbit liver (33) and mouse myeloma (34). Aside from universal nucleotides (35) and the anticodon these tRNAs have the following residues in common: U_4, A_9, C_{11}, C_{13}, G_{22}, G_{24}, m^7G46, and A_{73}. However, while sp.chl. $tRNA^{Met}_{m}$ contains the residues A_1, A_{10}, C_{16}, Ψ_{25}, U_{32}, C_{38}, G_{39}, A_{45}, A_{57}, A_{63} and U72, all of the non-organelle $tRNA^{Met}_{m}$ have, in each of these positions, a residue which is the same in all of these tRNAs but which differs from the corresponding residue in the sp.chl. $tRNA^{Met}_{m}$. Further sequences of methionine elongator tRNAs, especially from organelles, are needed before it can be determined if some of these positions are indeed characteristic of organelle as compared to non-organelle methionine elongator tRNAs.

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