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The nucleotide sequence of spinach chloroplast tryptophan transfer RNA

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ABSTRACT

Spinach chloroplast $tRNA^{Trp}$, purified by column chromatography and two-dimensional gel electrophoresis, has been sequenced using in vitro labeling techniques. The sequence is : pG-C-G-C-U-C-U-U-A-G-U-U-C-A-G-U-U-C-Gm-G-D-A-G-A-A-C-m^2G-ψ-G-G-G-ψ-C-U-C-C-A-A-A-A-C-C-C-G-A-U-G-N-C-G-U-A-G-G-T-ψ-C-A-A-G-U-C-C-U-A-C-A-G-A-G-C-G-U-G-C-C-A_{OH}.*

Like the E.coli suppressor tRNA psu+UGA which translates both the opal terminator codon U-G-A and the tryptophan codon U-G-G, spinach chloroplast $tRNA^{Trp}$ has C-C-A as an anticodon and contains an A-U pair in the D-stem.

INTRODUCTION

Chloroplasts contain their own DNA genome and a complete apparatus for protein synthesis, including ribosomes, tRNAs and aminoacyl-tRNA synthetases. While chloroplasts are dependent on the nucleo-cytoplasmic system for a number of functions which necessitate import of proteins from the cytoplasm, they seem to be autonomous with respect to their RNA complement. Hybridization studies have shown that chloroplast DNA from various plant species codes for the chloroplast ribosomal RNAs (1). The majority of the chloroplast tRNAs from spinach (2) and from Euglena (3) hybridize with the chloroplast DNA. Relatively few chloroplast tRNAs have been sequenced and so far little information is available concerning codon usage in chloroplast messenger RNAs, although the sequence of the gene coding for the large subunit of ribulose 1,5 bisphosphate carboxylase on maize chloroplast DNA has recently been determined (4).

Recently, sequence studies have shown that an altered genetic code is used in the human (5) and yeast (6) mitochondrial genomes since, for example, the opal codon U-G-A, normally used for termination of protein synthesis in procaryotes and in the cyto-

plasm of eucaryotes, codes for tryptophan. The sequence of yeast mitochondrial $tRNA^{Trp}$ (7) shows that this tRNA has an anticodon U^{*}-C-A which should allow pairing with both the usual tryptophan codon U-G-G and the opal terminator codon U-G-A. To determine whether chloroplast $tRNA^{Trp}$ is likely to read the opal terminator codon, we have determined the sequence of the spinach chloroplast $tRNA^{Trp}$.

MATERIALS AND METHODS

Purification of spinach chloroplast $tRNA^{Trp}$

Crude tRNA, extracted from spinach (*Spinacia oleracea*) leaves using standard procedures (8) was fractionated on a BD-cellulose column at pH 7.4 (using a linear NaCl gradient from 0.3 to 1.5 M). Spinach chloroplast $tRNA^{Trp}$ was identified by aminoacylation with an *E.coli* enzyme extract (2). The fractions containing tryptophan accepting activity were then concentrated. After preparative aminoacylation, $|^{14}C|$-tryptophanyl-$tRNA^{Trp}$ was chromatographed on a BD-cellulose column at pH 4.5 and eluted by an ethanol gradient from 0% to 20% containing 1.1 M NaCl. Polyacrylamide gel electrophoresis was used as a final purification step (9,2).

Sequencing techniques

The sequence of chloroplast $tRNA^{Trp}$ was determined primarily using the technique described by Stanley and Vassilenko (10) with the modifications reported previously (11). 2 µg of $tRNA^{Trp}$ was partially hydrolyzed in bidistilled water at 80° for 3 min. After 5' $|^{32}P|$-labeling under standard conditions (12) using 2 units of T_4 polynucleotide kinase (P.L. Biochemicals) and 300 µCi $|^{32}P|$ ATP 2000 Ci/mmole (Amersham) the fragments were separated on a thin 15% polyacrylamide slab gel (13). Each fragment was completely digested with nuclease P_1 (0.1 µg nuclease/$A_{260\ nm}$ carrier tRNA in 50 mM ammonium acetate buffer pH 5.3) 30 min at 37°C and the 5' terminal nucleotide was identified by thin layer chromatography on cellulose plates (20 x 20 cm or 10 x 10 cm) (Schleicher & Schuell) in the solvents indicated in the legend to figure 1.

Labeling of the 3' terminus of chloroplast $tRNA^{Trp}$ was done using two standard techniques : i) after treatment with venom

phosphodiesterase, tRNA was labeled using α-$|^{32}P|$-ATP and tRNA nucleotidyltransferase (12) or ii) T_4 RNA ligase was used to add ^{32}pCp to the 3' end of the intact tRNA molecule (14). Read-off sequencing gels (15,16) were used to analyse the end labeled tRNA. Conditions for enzymatic digestions were those described previously (17).

Labeling of the 5' terminus of spinach chloroplast $tRNA^{Trp}$ was inefficient using standard techniques (12,18) but a number of 5' labeled degradation fragments of $tRNA^{Trp}$ were obtained using these procedures. The sequence of these fragments were determined by mobility shift analysis : a method involving partial hydrolysis of the end-labeled fragment using nuclease P_1, followed by separation of the partial digestion products by two-dimensional homochromatography. Fragments were also analysed on sequencing gels, using the conditions described for 3' end labeled tRNA.

RESULTS

The majority of the results used to determine the sequence of spinach chloroplast tRNA were obtained using the technique developed by Stanley and Vassilenko (10), with the modifications described in Materials and Methods. The 5'-labeled fragments obtained using this technique were separated by polyacrylamide gel electrophoresis (Fig. 1A). The 5' termini of the fragments were identified by thin layer chromatography. The results obtained gave the sequence from position 5 to 72 in the chloroplast $tRNA^{Trp}$. In Fig. 1B, the one-dimensional separation of the 5' termini of fragments corresponding to the D-stem and loop (positions 10 to 25) and to the anticodon region (positions 30 to 45) is shown. In addition, two-dimensional thin layer chromatography was used to identify modified nucleotides present in the tRNA. A number of fragments were also sequenced using mobility shift analysis (see Materials and Methods).

The sequence obtained using the Stanley-Vassilenko technique was confirmed and completed (residues 1-4, 19, and 73-76) by analysis of 3'-labeled $tRNA^{Trp}$ and 5'-labeled fragments of $tRNA^{Trp}$. The results obtained were in good agreement with those obtained by the Stanley and Vassilenko technique, except for

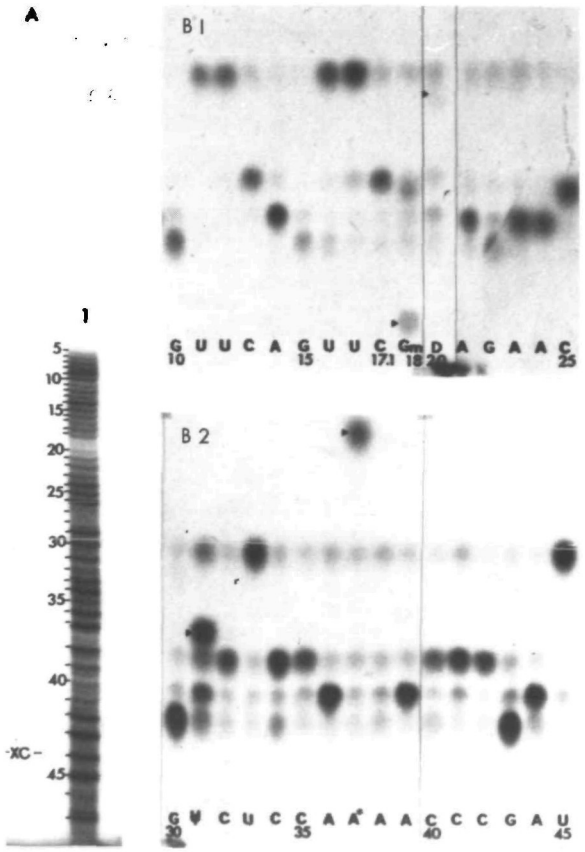

Fig. 1. Sequence analysis of spinach chloroplast $tRNA^{Trp}$ using the Stanley and Vassilenko technique.

A. One of the separations of 5'-$[^{32}P]$-labeled hot water hydrolysate of $tRNA^{Trp}$ on 15% polyacrylamide gel (40 x 30 cm x 0.5 mm), 8 M urea 45 mM Tris-borate pH 8.3 ; XC = xylene cyanol marker.

B1,B2. Fragments eluted from the polyacrylamide gel (1A) were totally digested with nuclease P_1. The 5' termini were separated on cellulose plates (20 x 20 cm) using the following solvent : 2-propanol: conc. HCl : H_2O 70:15:15 (V/V/V).

residues 26 and 27. When 3' end labeled $tRNA^{Trp}$ was analysed on sequencing gels, the fragments corresponding to these two residues comigrated as a result of band compression. This ambiguity was resolved by analysis of 5' end-labeled fragments of $tRNA^{Trp}$.

DISCUSSION

The structure of spinach chloroplast $tRNA^{Trp}$ is shown in Fig. 2. The sequence contains the invariant and semi-invariant nucleotides present in the usual cloverleaf structure, but shows two features not found previously in other $tRNAs^{Trp}$: it contains a nucleotide in position 17.1 in the D-loop and lacks m^7G in the variable loop. The D-loop of spinach chloroplast $tRNA^{Trp}$ contains 9 nucleotides rather than 7 found in $tRNAs^{Trp}$ from eucaryotic cytoplasm (19,20,21) or 8 found in the D-loop of *E.coli* $tRNAs^{Trp}$ (22,23,24). All seven $tRNAs^{Trp}$ sequenced so far have m^7G in

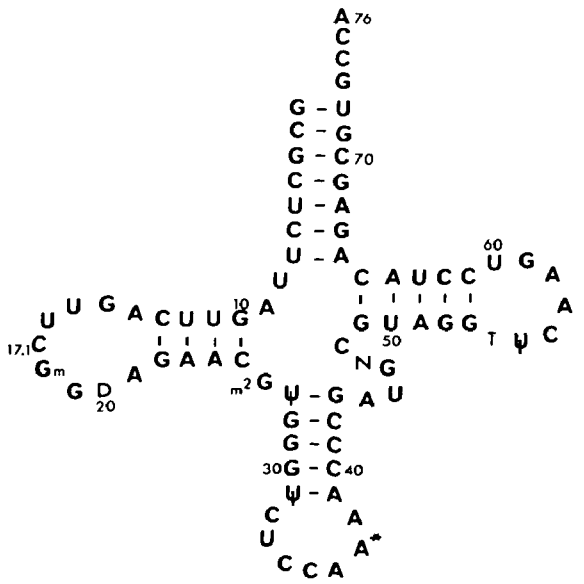

Fig. 2. Nucleotide sequence of spinach chloroplast $tRNA^{Trp}$
A^*37 : i^6A or ms^2i^6A
N47 : unidentified modified nucleotide

position 46 except an undermodified minor species of bovine liver $tRNA^{Trp}$ (21). Spinach chloroplast $tRNA^{Trp}$ contains an unmodified G instead of m^7G at position 46.

The anticodon of spinach chloroplast $tRNA^{Trp}$, C-C-A, corresponds to the usual tryptophan codon U-G-G and,theoretically, should not be able to decode the U-G-A opal terminator codon. In contrast, the yeast mitochondrial $tRNA^{Trp}$ has an anticodon U^{*}-C-A (7) which is capable, according to the wobble hypothesis, of base pairing with both U-G-G and U-G-A. However, this is not the only example of a $tRNA^{Trp}$ capable of decoding U-G-A since the $tRNA^{Trp}$ from an su+UGA strain of *E.coli* (CAJ64) suppresses a UGA mutation in lysozyme mRNA during cell-free protein synthesis (25). This $tRNA^{Trp}$, like the wild-type *E.coli* $tRNA^{Trp}$ has C-C-A as an anticodon. The sequence of the *E.coli* suppressor $tRNA^{Trp}$ only differs from that of the wild-type $tRNA^{Trp}$ by one nucleotide in the D-stem : the suppressor tRNA has a U11:A24 base pair, whereas the wild-type tRNA has U11:G24 (22). Both these $tRNAs^{Trp}$, like spinach chloroplast $tRNA^{Trp}$, have C-C-A as an anticodon, indicating that an altered anticodon is not necessary to read U-G-A. The sequence of spinach chloroplast $tRNA^{Trp}$ shows 68% homology with that of the *E.coli* suppressor tRNA psu+UGA and contains the same U11:A24 base pair. In view of these similarities, it is worthwhile to investigate whether spinach chloroplast $tRNA^{Trp}$ is capable of reading U-G-A in a cell-free protein synthesizing system.

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