Volume 10 Number 8 1982 Nucleic Acids Research

Nucleotide sequence of a spinach chloroplast proline tRNA

M.Francis, M.Kashdan, H.Sprouse, L.Otis and B.Dudock

Department of Biochemistry, State University of New York at Stony Brook, Stony Brook, NY 11794, USA

Received 21 October 1981; Revised and Accepted 17 March 1982

ABSTRACT

The nucleotide sequence of a spinach chloroplast proline tRNA (sp. chl. $tRNA^{pro}$) has been determined. This tRNA shows more overall homology to phage T4 proline tRNA (61% homology) than to eukaryotic proline tRNAs (53% homology) or mitochondrial proline tRNAs (36-49% homology). Sp. chl. $tRNA^{pro}$, like all other chloroplast tRNAs sequenced, contains a methylated GG sequence in the dihyrouridine loop and lacks unusual structural features which have been found in many mitochondrial tRNAs.

INTRODUCTION

Mitochondria and chloroplasts contain their own DNA and protein synthesizing systems. Protein synthesis in mitochondria shows significant differences from that occurring in prokaryotes and eukaryotic cytoplasm. For example specific changes in the genetic code have been observed (1-3) and a variety of unusual tRNA and tRNA genes studied (2-4). Protein synthesis in chloroplasts is not as well characterized as in mitochondria. As part of a study of protein synthesis in chloroplasts we have determined the nucleotide sequence of a spinach chloroplast proline 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 sequence determination and hybridization have been described previously (6-8)

RESULTS

Purification of tRNA The pure $tRNA^{pro}$ used for sequence analysis and hybridization studies was obtained by a three step chromatographic procedure consisting first of a benzoylated DEAE cellulose column run at pH 7.6. Fractions 209-231 of this column (see Fig. 1 of reference 9) were pooled and further purified on an RPC-5 column at pH 4.5. Finally, $tRNA^{pro}$ was purified on an RPC-5 column at pH 7.5 to homogeneity as judged by a

0305-1048/82/1008-2755$ 2.00/0

single band on a denaturing 20% polyacrylamide gel, stained with ethidium bromide (data not shown). This tRNA was shown by subsequent sequence analysis and aminoacylation (with E. coli synthetase) to be a proline tRNA.

Sequence Analysis The nucleotide sequence of sp. chl. $tRNA^{pro}$ was determined using formamide fragment analysis, RNA sequence gels and mobility shifts. The formamide fragment analysis method (10,11) provided the most sequence information of the three methods employed and allowed an unambiguous assignment of residues 3 to 74 with the exception of residue G_{10} for which there was no corresponding band in the formamide generated "ladder". Missing bands, compression effects and other anomalies have occasionally been observed in the formamide fragment procedure (6,7,11) and underscore the need for confirmatory sequence procedures. Residue 10 was identified as G on the basis of an RNase T1 band on several RNA sequence gels. However, since RNA sequence gels cannot distinguish between a G and a modified G, and since this residue was not detected on the formamide fragment analysis method, we conclude that residue 10 is either a G or a modified G.

The dinucleotide GmG in the dihydrouridine loop and all other modified nucleotides obtained using formamide fragment analysis were identified as described previously (7). RNA sequence gels (6,12-14) were used to analyze both 5'-^{32}P and 3'-^{32}P labeled sp. chl. $tRNA^{pro}$. These gels confirmed the assignment of residues 1-75. The 3' end of the $tRNA^{pro}$ was also determined by mobility shift procedures, performed as described (15).

Hybridization of Spinach Chloroplast $tRNA^{pro}$ The sp. chl. [3'-^{32}P] $tRNA^{pro}$ was hybridized to restriction fragments of sp. chl. DNA which were generated by the enzymes SalI and KpnI respectively using methods previously described (7,8,16-18). The sp. chl. [3'-^{32}P]$tRNA^{pro}$ hybridized to a 13.8 megadalton SalI fragment and to a 13.8 megadalton KpnI fragment, on a part of the sp. chl. genome where other tRNAs are known to hybridize (19,20).

DISCUSSION

The nucleotide sequence of sp. chl. $tRNA^{pro}$ is shown in Fig. 1. This tRNA contains 77 residues, the same size as phage T4 $tRNA^{pro}$ (21), both of which are larger than the eukaryotic proline tRNAs from mouse and chicken (21) (75 residues) and mitochondrial proline tRNAs from yeast (22) (75 residues) and human cells (3) (71 residues). In addition sp. chl. $tRNA^{pro}$ shows more overall homology to phage T4 $tRNA^{pro}$ (61% homology) than to eukaryotic proline tRNAs (53% homology) or mitochondrial $tRNA^{pro}$ (36-49% homology). Unfortunately no prokaryotic $tRNA^{pro}$ has yet been sequenced.

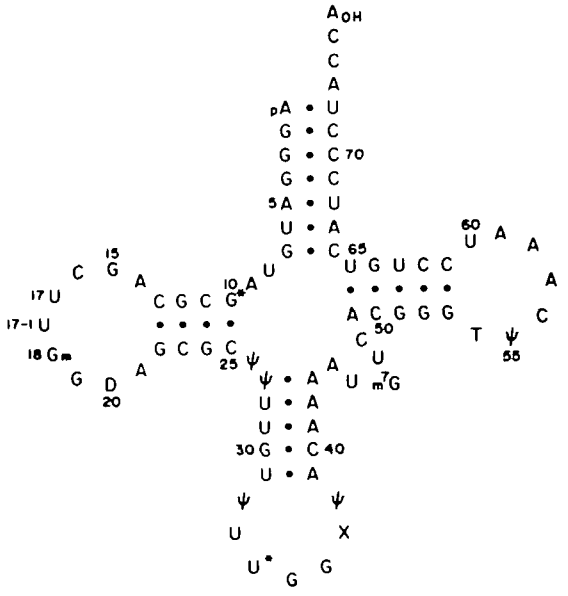

Figure 1. Nucleotide sequence of spinach chloroplast proline tRNA
Residue G^{*}_{10} may be a modified G.

Sp. chl. $tRNA^{pro}$ contains a methylated GG sequence in the dihydrouridine loop, a feature found in all chloroplast tRNAs sequenced to date. Sp. chl. $tRNA^{pro}$ contains an unknown modified uridine, denoted as U*, in the wobble position of the anticodon (residue 34). This same residue is also found in sp. chl. $tRNA^{val}$ and its characteristics have been discussed (23). Sp. chl. $tRNA^{pro}$ also contains an unknown modified residue at position 37 denoted as X. Residue X appears to be similar but not identical to $t^{6}A$. Residue X migrates as $pt^{6}A$ in the two-dimensional system of Silberklang, et al. (15) but migrates slightly slower than $pt^{6}Ap$ in the ammonium formate system of Gupta and Randerath (11). Authentic $t^{6}A$ was obtained from bovine liver $tRNA^{met}_{i}$.

A comparison of the sequences of the six known proline tRNAs suggests that certain residues (numbered as in Fig. 1) may be of interest as follows: A) G_3 is present in the three organelle $tRNA^{pro}$ while C_3 is present in the three non-organelle $tRNA^{pro}$; B) G_{10} followed by a pyrimidine at residue 11 is present in all $tRNA^{pro}$; C) G_{15} is present in all except mitochondrial $tRNA^{pro}$; D) all $tRNA^{pro}$ have pyrimidines at residues 27 and 28, a U or

modified U at residue 32 and purines at residues 42 and 43; E) non-eukaryotic $tRNA^{pro}$ have A_{73} while eukaryotic $tRNA^{pro}$ have C_{73}. These observations are based upon only six proline tRNAs and sequence analysis of additional proline tRNA will be of interest.

ACKNOWLEDGEMENTS

We thank H. Donis-Keller, J. Dunn and H. Vreman. This investigation was supported in part by grants from NIH (GM-25254) and NSF (PCM-7922751).

REFERENCES

1. Hall, B.D. (1979) Nature 282, 129-130.
2. Li, M. and Tzagoloff, A. (1979) Cell 18, 47-53.
3. Anderson, S., Bankier, A.T., Barrell, B.G., deBruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R. and Young, I.G. (1981) Nature 290, 457-465.
4. Arcari, P. and Brownlee, G.G. (1980) Nucleic Acids Res. 8, 5207-5212.
5. deBruijn, M.H.L., Schreier, P.H., Eperon, I.C., Barrell, B.G., Chen, E.Y., Armstrong, P.W., Wong, J.F.H. and Roe, B.A. (1980) Nucleic Acids Res. 8, 5213-5222.
6. Pirtle, R., Kashdan, M., Pirtle, I. and Dudock, B. (1980) Nucleic Acids Res. 8, 805-815.
7. Kashdan, M.A., Pirtle, R.M., Pirtle, I.L., Calagan, J.L., Vreman, H.J. and Dudock, B.S. (1980) J. Biol. Chem. 255, 8831-8835.
8. Calagan, J.L., Pirtle, R.M., Pirtle, I.L., Kashdan, M.A., Vreman, H.J. and Dudock, B.S. (1980) J. Biol. Chem. 255, 9981-9984.
9. Delihas, N., Andersen, J., Sprouse, H.M., Kashdan, M. and Dudock B. (1981) J. Biol. Chem. 256, 7515-7517.
10. Stanley, J. and Vassilenko, S. (1978) Nature 274, 87-89.
11. Gupta, R.C. and Randerath, K. (1979) Nucleic Acids Res. 6, 3443-3458.
12. Donis-Keller, H., Maxam, A. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538.
13. Sanger, F. and Coulson, A.R. (1978) FEBS Letters 87, 107-110.
14. Simoncsits, A., Brownlee, G.G., Brown, R.S., Rubin, J.R. and Guilley, H. (1977) Nature 269, 833-836.
15. Silberklang, M., Gillum, A.M. and RajBhandary, U.L. (1979) in Methods in Enzymology, Moldave, K. and Grossman, L., eds. Vol. 59, pp. 58-109.
16. Whitfeld, P.R., Herrmann, R.G. and Bottomley, W. (1978) Nucleic Acids Res. 5, 1741-1751.
17. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
18. Heckman, J.E. and RajBhandary, U.L. (1979) Cell 17, 583-595.
19. Steinmetz, A., Mubumbila, M., Keller, M., Burkard, G., Weil, J.H., Driesel, A.J., Crouse, E.J., Gordon, K., Bohnert, H.J. and Herrmann, R.G. (1978) in Chloroplast Development (Akoyunoglou, G. and Argyroudi-Akoyunoglou, J.H., eds.) pp. 573-580 Elsevier/North Holland.
20. Driesel, A.J., Crouse, E.J., Gordon, K., Bohnert, H.J., Herrmann, R.G., Steinmetz, A., Mububmila, M., Keller, M., Burkard, G. and Weil, J.H. (1979) Gene 6, 285-306.
21. Gauss, D.H. and Sprinzl, M. (1981) Nucleic Acids Res. 9, r1-r22.
22. Newman, D., Phem, H.D., Underbrink-Lyon, K. and Martin, N.C. (1980) Nucleic Acids Res. 8, 5007-5016.
23. Sprouse, H.M., Kashdan, M., Otis, L. and Dudock, B. (1981) Nucleic Acids Res. 9, 2543-2547.