The nucleotide sequences of a cytoplasmic and a chloroplast tRNA^{Tyr} from *Scenedesmus obliquus*

Gaynor A.Green* and David S.Jones+

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK

Received 17 December 1984; Revised and Accepted 12 February 1985

ABSTRACT

The nucleotide sequences of two species of tyrosine accepting tRNA from the eukaryotic green alga Scenedesmus obliquus have been determined. The sequence of the cytoplamic tRNA^{Tyr} is: pC-C-C-C-U-U-G-U-A-G-C-U-C-A-G-D-D-Gm-G-C -A-G-A-G-C-m^{2}G-G-G-A-G-A-C-U-G-ψ-A-m^{1}G-A-ψ-C-U-C-U-A-G-m^{7}G-N-C-A-C-U-G-G-T-ψ-C-G-m^{1}A-A-U-C-C-G-G-U-C-G-A-G-G-G-G-A-C-C-A-OH and that of the chloroplast tRNA^{Tyr} is: pG-G-G-C-C-G-A-U-G-C-C-G-A-A-G-U-U -Gm-G-D-U-A-A-U-G-G-G-G-C-G-G-A-ψ-U-G-U-A-(i^{6}A/ms^{2}i^{6}A)-A-ψ-C-C-G-C-U-G-G-U-U-A-C-G-C-C-U-A-C-G-U-U-G-G-T-ψ-C-G-A-A-U-C-C-G-A-C-U-C-G-G-C-C-C-A-C-C-A-OH. This is the first chloroplast tRNA^{Tyr} species to be sequenced.

INTRODUCTION

Previous studies on transfer RNA from the unicellular eukaryotic green alga, Scenedesmus obliquus, have been confined to the methionine accepting species. The sequence or partial sequence, of four species have been reported, namely, the cytoplasmic initiator [1] and elongator [2], and the chloroplast initiator [3] and elongator [4]. Our studies on the structure of tRNAs from S. obliquus have continued, and here we report the sequence determination of two tyrosine accepting species, one of cytoplasmic and one of chloroplast origin. As far as we are aware the only other plant tRNA^{Tyr} sequences which have been reported previously are those of wheat germ [5] and two tyrosine accepting suppressor tRNAs from tobacco [6]. No chloroplast tRNA^{Tyr} sequences have been reported to date, except for a few from higher plants which have been deduced from their gene sequences [7, 8, 9]. Only by sequencing the tRNAs can the full structures (including modified nucleosides) be determined. Both the cytoplasmic and chloroplast tRNA^{Tyr} species show interesting modification patterns.

MATERIALS AND METHODS

T_4 RNA ligase was obtained from PL Biochemicals Inc. and 5'-[^{32}P] pCp was either prepared according to the method of Silberklang et al. [10] or purchased at

2000-3000 Ci.mmol^{-1} from Amersham International plc. Otherwise, chemicals, enzymes, radiochemicals and chromatographic materials were obtained or prepared as described previously [1]. Total crude tRNA was isolated from S. obliquus by the method of Kirby [11] and the individual tRNA species were purified as described elsewhere [12].

The tRNAs were characterised by aminoacylation studies, and hybridisation of the respective [^{32}P]-labelled tRNA species to S. obliquus DNA fractionated by CsCl-density-gradient centrifugation [13].

The primary sequences were deduced using a combination of [^{32}P]-post-labelling techniques as described previously [1]. These were the 'limited hydrolysis' method [14], 'direct read-off' gels [15] and 'mobility shift' analysis [10]. In addition 'direct read-off' gels and 'mobility shift' analyses were performed on 3'-[^{32}P]-labelled tRNAs. The 5'- and 3'-terminal bases were determined as described previously [3], and modified nucleosides were identified by the methods of Nishimura [16] and Silberklang et al. [10].

RESULTS AND DISCUSSION

Fractionation of S. obliquus tRNA on BD-cellulose followed by RPC-5 chromatography separated three peaks of tyrosine accepting activity; $tRNA_1^{Tyr}$, $tRNA_{2a}^{Tyr}$ and $tRNA_{2b}^{Tyr}$. Hybridisation of these [^{32}P]-labelled species to S. obliquus DNA fractionated by CsCl-density-gradient centrifugation showed that $tRNA_1^{Tyr}$ is of nuclear origin, whilst $tRNA_{2a}^{Tyr}$ and $tRNA_{2b}^{Tyr}$ are of chloroplast origin. Polyacrylamide gel electrophoresis showed $tRNA_{2a}^{Tyr}$ and $tRNA_{2b}^{Tyr}$ to be of much higher MW than $tRNA_1^{Tyr}$. Also $tRNA_{2b}^{Tyr}$ ran just slightly slower than $tRNA_{2a}^{Tyr}$.

A combination of post-labelling sequencing techniques allowed the sequences of cytoplasmic $tRNA_1^{Tyr}$ and chloroplast $tRNA_{2b}^{Tyr}$ from S. obliquus to be determined unambiguously. Each residue was identified by at least two methods or experiments.

The structures of a cytoplasmic and chloroplast $tRNA^{Tyr}$ species from S. obliquus are shown in Fig. 1a and 1b respectively. As might be expected there is little homology (51%) between these two sequences and in particular it can be seen that they each belong to a different class of 'extra arm' structures. The short cytoplasmic one (5 residues) conforming to those of other cytoplasmic $tRNA^{Tyr}$ species and the long chloroplast one (15 residues) showing a typical prokaryotic-like $tRNA^{Tyr}$ character.

Q.-base (in the first position of the anticodon) has been found in several, but not all, of the tRNAs whose cognate codons are NA^{U}/C, namely $tRNA^{Asn}$, $tRNA^{Asp}$, $tRNA^{His}$ and $tRNA^{Tyr}$ [17]. So far it has been found in eubacterial and some

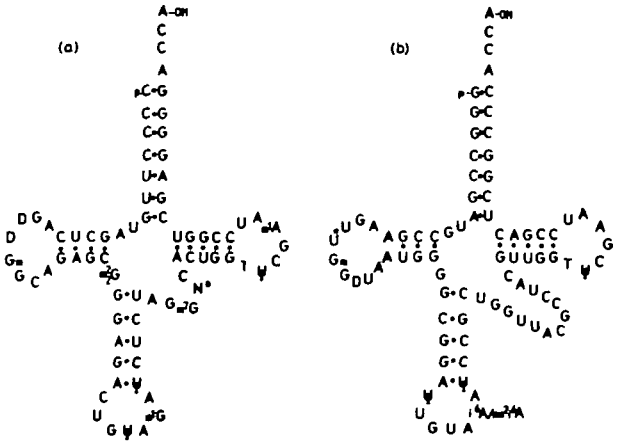

Fig.1 Clover leaf structures of (a) Cytoplasmic $tRNA_1^{Tyr}$ and (b) chloroplast $tRNA_{26}^{Tyr}$ from S. obliquus. N*, unidentified modified nucleoside; U*, unknown uridine modification.

mammalian tRNAs [17] and also in wheat germ $tRNA^{Tyr}$ [5]. However, it is not present in $tRNA^{Tyr}$ from adult wheat (i.e. wheat leaves) [5] nor in $tRNA^{Tyr}$ from tobacco [6]. It will be seen that Q is absent from both the cytoplasmic and the chloroplast $tRNA^{Tyr}$ species from S. obliquus. In this respect the chloroplast $tRNA^{Tyr}$ species differs from eubacterial $tRNA^{Tyr}$ species. Although the function of Q is not fully understood [18] it has been shown that Q-containing $tRNA^{Tyr}$ species from Drosophila melanogaster [19] and wheat germ [5] do not possess the suppressor property of their hypomodified $tRNA_{G\psi A}^{Tyr}$ species which are able to read through a terminator codon (UAG) during TMV expression. However, the apparent random way in which Q is present or not in tRNAs from different organisms means that it is difficult to make any generalisation as to its function.

Cytoplasmic $tRNA_1^{Tyr}$

S. obliquus cytoplasmic $tRNA^{Tyr}$ shows 77% homology with wheat $tRNA^{Tyr}$, and 79% and 75% homology respectively with the two cytoplasmic $tRNA^{Tyr}$ species from tobacco [6]. The major regions of variation between S. obliquus and those of other plant tRNAs are in the amino acid and anticodon stems. There is slightly less homology with X. laevis $tRNA^{Tyr}$ (74%) and less still with yeast (63%) [17].

The pattern of nucleoside modifications in S. obliquus cytoplasmic $tRNA^{Tyr}$ is typically eukaryotic. The nucleoside following the third residue of the anticodon (i.e. N_{37}) is m^1G, which is present also in $tRNA^{Tyr}$ from wheat, tobacco and X.

laevis. Eubacterial and yeast $tRNA^{Tyr}$ species have a modified A at this position. (It is interesting to note that yeast $tRNA^{Tyr}$ is also similar to the eubacterial species in having a long extra arm). Like other eukaryotes (including yeast) S. obliquus cytoplasmic $tRNA^{Tyr}$ has Ψ as the middle base of the anticodon. In all there are 12 modifications in S. obliquus $tRNA^{Tyr}$, 9 of which are different. The modification at position 47 is unidentified. The electrophoretic and chromatographic properties of this nucleoside are distinct from those reported for any other modified nucleoside, including acp^3U which is present at position 47 in wheat and tobacco $tRNA^{Tyr}$. Interestingly, this unknown modified nucleoside is also present at postion 47 in S. obliquus cytoplasmic $tRNA^{Met}_m$ [2] and $tRNA^{Phe}$ (Green and Jones unpublished).

Chloroplast $tRNA^{Tyr}_{2b}$

This S. obliquus tRNA species is typically prokaryotic-like in character, although its overall homology with E. coli $tRNA^{Tyr}$ is not high (64%). It has a low level of base modification (a total of 8 modifications of 6 types), of which all but two are derivatives of U. This tRNA sequence conforms to the general rule for prokaryotic tRNAs which predicts the residue at position 37 [16]. Where U is the first base of the codon, an isopentenyl derivative of A would be expected at position 37. In chloroplast $tRNA^{Tyr}_{2b}$ N_{37} appears to be a mixture of i^6A and ms^2i^6A. The residue at position 17 has not been conclusively identified. Electrophoretically this nucleotide runs just faster than U and therefore it is likely to be a derivative of U. However its chromatographic properties are distinct from any U derivative reported to date.

Although this is the first chloroplast $tRNA^{Tyr}$ species to be sequenced comparisions with other chloroplast $tRNA^{Tyr}$ species are possible since tRNA sequences can be deduced from their gene structures. Thus S. obliquus chloroplast $tRNA^{Tyr}$ shows 82% homology with Vicia faba [8] and spinach [9] chloroplast $tRNA^{Tyr}$ species and 77% homology with the Euglena species [7]. Although, as expected, each of these chloroplast $tRNA^{Tyr}$ species have long extra arms, interestingly they all differ in size. The extra arm of the S. obliquus species has 13 residues whereas those from V. faba and spinach, have 15 residues, and E. gracilis has 12 residues. The extra arm is the the region of least homology between the S. obliquus and these other chloroplast $tRNA^{Tyr}$ species.

Preliminary analysis of the second chloroplast species from S. obliquus, $tRNA^{Tyr}_{2a}$, suggests that its sequence resembles very closeley that of $tRNA^{Tyr}_{2b}$. Since the 5'- and 3' - end residues of these two tRNAs are the same, it is likely that the difference in migration on polyacrylamide gel electrophoresis is the result of a nucleoside modification rather than a size difference. It is probable that these two chloroplast $tRNA^{Tyr}$ species are derived from the same gene.

ACKNOWLEDGEMENT

G. A. Green acknowledges the receipt of a S.E.R.C. research studentship.

*Present address: Institut de Biologie Moleculaire et Cellulaire du CNRS, Universite Louis Pasteur, 15 rue Rene Descartes, F-67084 Strasbourg, France.

+To whom correspondence should be addressed.

REFERENCES

1. Olins, P. O. and Jones, D. S. (1980) Nucleic Acids Res. 8, 715-729.
2. McCoy, J. M. and Jones, D. S. (1983) International tRNA Workshop Hakone, Japan.
3. McCoy, J. M. and Jones, D. S. (1980) Nucleic Acids Res. 8, 5089-5093.
4. McCoy, J. M. and Jones, D. S. (1980) EMBO-FEBS tRNA Workshop, Strasbourg.
5. Beier, H., Barciszewska, M. and Sickinger, H-D. (1984) EMBO J. 3, 1091-1096.
6. Beier, H., Barciszewska, M., Krupp, G. Mitnacht, R. and Gross, H. J. (1984) EMBO J. 3, 351-356.
7. Sprinzl, M. and Gauss, D. H. (1984) 'Compilation of sequences of tRNA genes' 8.Kuntz, M., Weil, J. H. and Steinmetz, A (1984) Nucleic Acids Res. 12, 5037-5047.
9. Holschuh, K., Bottomley, W and Whitfield, P. R. (1984) Plant Molec. Biol. 3 313-317.
10. Silberklang, M., Gillam, A. M. and RajBhandary, U. L. (1979) Methods Enzymol. 59, 58-109.
11. Kirby, K. S. (1965) Biochem. J. 96, 266-269.
12. Green, G. A. and Jones, D. S. (1984) Biochem. Soc. Trans. 12, 468-470.
13. Pryke, J. A., Cranney, M. K. and Jones, D. S. (1979) Plant Science Letters 16, 125-128.
14. Stanley, J. and Vassilenko, S. (1978) Nature 274, 87-89.
15. Donis-Keller, H., Maxam, A. M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538.
16. Nishimura, S. (1972) Progr. Nucleic Acid Res. Mol. Biol. 12, 49-85.
17. Sprinzl, M. and Gauss, D. H. (1984) 'Compilation of tRNA Sequences', Nucleic Acids Res. 12, r1-r57.
18. Nishimura, S. (1983) Progr. Nucleic Acid Res. Mol. Biol. 28, 49-73.
19. Bienz, M. and Kubli, E. (1981) Nature 294, 188-190.