PRIMARY STRUCTURE OF BEAN CHLOROPLASTIC tRNA^{Phe}

Comparison with euglena chloroplastic tRNA^{Phe}

P. GUILLEMAUT and G. KEITH
Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue Descartes, 67084 Strasbourg Cédex, France

Received 21 September 1977

1. Introduction

The nucleotide sequence of *Euglena gracilis* chloroplast (chls) tRNA^{Phe} has been published [1]. This is the first organellar tRNA so far sequenced. To date, in higher plants, only cytoplasmic tRNAs^{Phe} had been sequenced, and it was shown that the tRNAs^{Phe} from wheat germ [2], pea germ [3] and a minor species (20%) of lupine seeds [4] have identical primary structures. It was therefore of great interest to compare the algal chls tRNA^{Phe} (*Euglena gracilis*), to the major chls tRNA^{Phe} from a higher plant *Phaseolus vulgaris*, which has been purified and whose overall nucleoside composition has been established recently [5].

In this work we report the analyses of the exhaustive T_1 RNAase and pancreatic RNAase digestion products. We also report the study of the primary structure of two large fragments (1/3 and 2/3) derived from chemical cleavage of the tRNA^{Phe} at m^{7}G. The primary structure is deduced and discussed.

2. Materials and methods

The purification of the major species of bean chls tRNA^{Phe} (peak 1) was described [5].

The T_1 RNAase and pancreatic RNAase digestion products were postlabelled using polynucleotide kinase from T_4-infected *E. coli* by transfer of ^{32}P from [γ-^{32}P]ATP to the 5'OH end of the 3'dephosphorylated oligonucleotides or tRNA, as described [6]. Polynucleotide kinase was prepared starting from commercial T_4-infected *E. coli* (purchased at New England Enzyme Center, Boston, USA) according to [7,8]; [γ-^{32}P]ATP was prepared according to [9].

For the postlabelling of oligonucleotides, the conditions were as described [6], except that 0.005 A_{260} units of digest in 1 μl and 1.5 nmol [γ-^{32}P]ATP (50–200 Ci/mmol) in 5 μl were used.

P_1 RNAase (P. L. Biochemicals) digestion, two-dimensional cellulose acetate electrophoresis followed by homochromatography on DEAE-cellulose TL were as described [10–12].

Cleavage of phosphodiester bond adjacent to m^{7}G was performed as described [13]. The products were separated by polyacrylamide gel electrophoresis, and the fragments recovered from the gel and analysed.

The determination of the 5'-end pancreatic specific oligonucleotide of tRNA^{Phe} was done by labelling the 5'OH-end of dephosphorylated tRNA^{Phe}, purification on polyacrylamide gel electrophoresis, recovery of 5'-labelled tRNA^{Phe} from the gel, and pancreatic RNAase digestion, followed by cochromatography with cold pG–Cp and pG–Up, and analysis of the ^{32}P-labelled end nucleotide.

3. Results

3.1. *Analysis of pancreatic RNAase digestion products*

Bean chls tRNA^{Phe} differs only slightly from Euglena chls tRNA^{Phe} as shown in fig.1A,B and table 1 Only four differences appear on the fingerprints:

(i) Two additional spots pG–G–C and pA–G–A–G–G A–C appear in bean chls tRNA^{Phe} hydrolysate.

Table 1
Sequence of oligonucleotides present in fingerprints of RNAase digests of bean and euglena chls $tRNAs^{Phe}$

Spot No.	Pancreatic RNAase digest (fig.1A,B)			Spot No.	T_1 RNAase digest (fig.1C,D)		
	Oligonucleotide	Bean	Euglena		Oligonucleotide	Bean	Euglena
1	A–C	+	+	1	C–A–C–C–A	+	+
2	m^7G–acp^3U	+	–	1[b]	C–G		+
2[a]	m^7G–Py*	–	+	2	A–G	+	+
2[b]	G–C	–	+	3	C–A–G	+	–
3	A–G–C	+	+	4	U–C–G	+	–
4	A–A–A–U	+	+	4[b]	C–U–G	–	+
5	G–U	+	+	5	D–A–G	+	–
6	G–G–C	+	–	5[a,b,c]	Gm–U*–A–G	–	+
7	A–G–U	+	–	6	A–C–U–G	+	+
7[c]	A–G–D	–	+	7	C–U–C–A–G	+	+
8	A–G–T	+	+	8	A–U–A–G	+	+
9	A–G–A–G–C	+	+	9	U–m^7G–acp^3U–C–A–C–C–A–G	+	–
10	Gm–G–D	+	–	9[a]	U–m^7G–U*–C–A–C–C–A–G	–	+
10[a,c]	G–Gm–U*	–	+	9[b,c]	D–U–G	–	+
11	G–G–U	+	+	10	U–D–Gm–G	+	–
12	A–G–A–G–G–A–C	+	–	11	U–U–C–C–U–G	+	–
12[b]	G–G–A–G–G–A–C	–	+	11[b]	U–U–C–C–U–A–G	–	+
13	G–A–A–ms^2i^6A–A–ψ(d)	+	–	12	A–A–ms^2i^6A–A–ψ–C–C–U–C–G	+	–
13[a]	G–A–A–A*–A–ψ	–	+	12[a,b]	A–A–A*–A–ψ–C–C–U–U–G	–	+
14	G–G–G–A–U	+	+	13	T–ψ–C–A–A–A–U–C–U–G	+	+

[a] The minor bases Py*, U* and A* in Euglena chls $tRNA^{Phe}$ are probably acp^3U, D and ms^2i^6A, respectively [1]
[b] These oligonucleotides contain some of the minor sequence differences found between the two chls $tRNAs^{Phe}$
[c] Post-transcriptional modification differences found between the two $tRNAs^{Phe}$
[d] Spots 13 and 13[a], despite identical sequence, were found at different positions on the respective fingerprints (fig.1C,D)

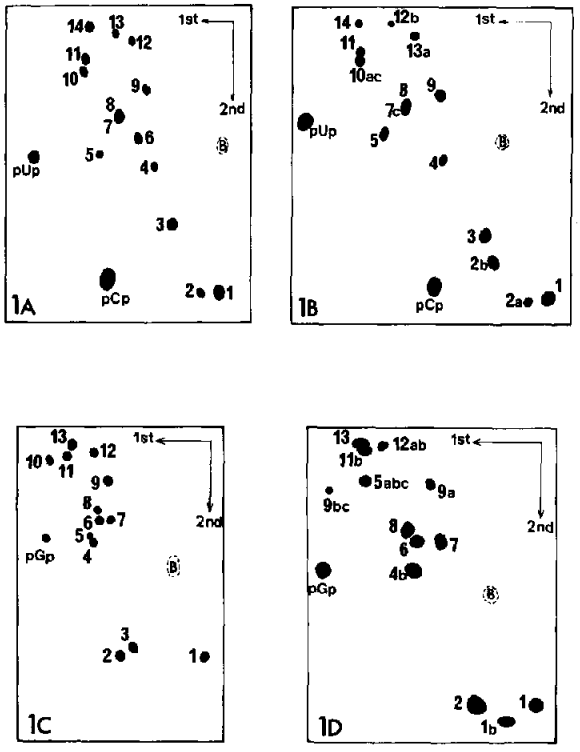

Fig.1. Fingerprints of 5′ ^{32}P-labeled oligonucleotides obtained from pancreatic RNAase digestion (1A,B) and T_1 RNAase digestion (1C,D). Fig.1B and 1D: Euglena chls $tRNA^{Phe}$ [1]. Fig.1A and 1C: bean chls $tRNA^{Phe}$. Electrophoresis in the first dimension was on cellulose acetate pH 3.5, and in the second dimension was on DEAE paper, 7% formic acid. 'B' surrounded by dots is a blue dye marker. The presence of pGp, pCp and pUp in these fingerprints is due to the presence of G-cyclic-p in T_1 RNAase digests and of C-cyclic-p and U-cyclic-p in pancreatic RNAase digests which are phosphorylated by polynucleotide kinase. For the oligonucleotide sequences, see table 1.

(ii) pG–G–A–G–G–A–C from Euglena is not present in the bean chls $tRNA^{Phe}$ fingerprint.

(iii) A fourth difference concerns the absence of pG–C in the bean chls $tRNA^{Phe}$ digest.

Four other differences are found:

(i) The Euglena chls $tRNA^{Phe}$ digest contains two times more pA–G–C than the bean chls $tRNA^{Phe}$ digest.

(ii) Spot (10) contains pGm at its 5′-end giving the sequence pGm–G–D (this is different in Euglena where Gm is located in the second position of that trinucleotide giving pG–Gm–U*.

(iii) pA–G–D from Euglena chls $tRNA^{Phe}$ is replaced by pA–G–U in bean chls $tRNA^{Phe}$.

(iv) The bean chls $tRNA^{Phe}$ contains two pG–U instead of only one in Euglena chls $tRNA^{Phe}$.

The 5′-end of bean chls $tRNA^{Phe}$ was determined as described under Materials and methods. First the ^{32}P-labelled 5′-end oligonucleotide obtained by pancreatic RNAase digestion comigrates with cold pG–Up on DEAE-cellulose paper electrophoresis in 7% formic acid. It yields [^{32}P]G when submitted to venom phosphodiesterase digestion, thus the 5′-end sequence is pG–U.

3.2. *Analysis of T_1 RNAase digestion products*

The fingerprint drawings shown in fig.1C,1D and table 1 give the differences between the T_1 RNAase digests of bean and Euglena chls $tRNA^{Phe}$.

The following oligonucleotides: pC–G; pC–U–G; pD–U–G; pGm–U*–A–G; pU–U–C–C–U–A–G; and pA–A–A*–A–ψ–C–C–U–U–G, found in Euglena chls $tRNA^{Phe}$, are absent in our digest, whereas the following oligonucleotides: pU–C–G; pC–A–G; pD–A–G; pU–D–Gm–G; pU–U–C–C–U–G; and pA–A–ms^2i^6A–A–ψ–C–C–U–C–G, found in bean chls $tRNA^{Phe}$, are absent in the Euglena $tRNA^{Phe}$. All other oligonucleotides were found in both fingerprints. It is interesting to point out that there is great similarity between the structures of these oligonucleotides. For instance, the few oligonucleotides from the pancreatic RNAase digest which were found to be different, can be associated two-by-two according to sequence homologies: pG–G–C and pA–G–C; pG–G–A–G–G–A–C and pA–G–A–G–G–A–C; pGm–G–D and pG–Gm–U*. Similarly the oligonucleotides from T_1 RNAase digests which were found to be different can be associated two-by-two. This will be discussed later.

3.3. *Analysis of two large fragments obtained by cleavage at the m^7G*

The tRNA was cleaved at the m^7G position and

Table 2
Analyses of two large fragments from bean chls $tRNA^{Phe}$ obtained by cleavage at the m^7G

Fragment	Spots from fig.1A present in pancreatic RNAase digest	Spots from fig.1C present in T_1 RNAase digest
3′-end (1/3) fragment	1–4–6–8–11	1–9*[a]–11–13
5′-end (2/3) fragment	3–5–7–9–10–12–13–14	2–3–4–5–6–7–8–10–12

[a]This oligonucleotide 9* corresponds to acp^3U–C–A–C–C–A–G since U–m^7G is lost by cleavage at the m^7G

the fragments separated on polyacrylamide gel as described in Materials and methods. The results of the analyses of oligonucleotides are described in table 2.

The smaller fragment contains the 3′-end sequence pC–A–C–C–A, whereas $pacp^3U$ was found at the 5′-end of the oligonucleotide $pacp^3U$–C–A–C–C–A–G which is part of pU-m^7G–acp^3U–C–A–C–C–A–G found in exhaustive T_1 RNAase digest. Among the remaining oligonucleotides, pA–G–T overlaps with $pacp^3U$–C–A–C–C–A–G and pT–ψ–C–A–A–A–U–C–U–G, whereas pG–G–U and pG–G–C overlap unambiguously in this fragment with pU–U–C–C–U–G and pC–A–C–C–A, respectively. These three overlaps give the sequence of the fragment from U–m^7G to the 3′-end C–A–C–C–A. Associating the different oligonucleotides two by two as above according to sequence similarities is indeed useful because we find that pU–U–C–C–U–G and pG–G–C (Euglena) occupy the same positions as pU–U–C–C–U–A–G and pA–G–C (bean), respectively.

The longer fragment contains two pG–U which correspond to the two end sequences of the fragment as shown below. Four large overlaps are established as follows: pU–D–Gm–G–D–A–G; pC–A–G–A–G–G–A–C–U–G; pG–G–G–A–U–A–G; and pG–A–A–ms^2i^6A–A–ψ–C–C–U–C–G; but it is not possible to deduce the entire sequence of the fragment.

Most of these overlaps and remaining oligonucleotides correspond to those found in Euglena chls $tRNA^{Phe}$. The only exceptions are underlined in the following sequences: pU–C–G; pU–D–Gm–G–U*; pA–G–A–G–G–A–C–U–G; pC–A–G; and pA–A–ms^2i^6A–A–ψ–C–C–U–C–G.

Nevertheless, it is quite impossible to assemble these overlapping sequences and oligonucleotides in a different arrangement than that found in the corresponding 5′-end 2/3 fragment of chls Euglena $tRNA^{Phe}$.

The overlap pU–D–Gm–G–D differs from pD–U–G–Gm–U*, only by the position of Gm and D, whereas pA–G–A–G–G–A–C–U–G differs only from pG–G–A–G–G–A–C–U–G by the G to A replacement of its 5′-terminal nucleotide and pA–A–ms^2i^6A–A–ψ–C–C–U–C–G differs from its Euglena counterpart by the C to U replacement of the nucleotide adjacent to G. These fragments occupy characteristic positions in Euglena chls $tRNA^{Phe}$. The first one is situated in the D loop in Euglena chls $tRNA^{Phe}$ with the G–Gm sequence corresponding to the invariant dinucleotide G_{18}–G_{19} found in all tRNAs working in protein synthesis. It is therefore quite unlikely that the situation should be different for the bean $tRNA^{Phe}$.

The other differences correspond in Euglena to the 5′- and 3′-side of the anticodon stem. Bean chls $tRNA^{Phe}$ has both fragments with exception of the 5′-end of pA–G–A–G–G–A–C–U–G and the nucleotide adjacent to G in pA–A–ms^2i^6A–A–ψ–C–C–U–C–G. It is therefore quite impossible to place these two fragments elsewhere in the molecule. Additional arguments come from the necessities to have the invariant U_{33}, to have a G–A–A anticodon (there is only one G–A–A triplet available inside the oligonucleotides from pancreatic RNAase digest) and G–A–A being followed by the hypermodified ms^2i^6A. Furthermore the fact that pC–A–G is found in bean and not in Euglena, whereas pC–G from the 5′-end of G–G–A–G–G–A–C is present in Euglena and absent in bean, provides an additional argument.

The trinucleotide pU–C–G, found in bean corresponds to pC–U–G in the Euglena sequence. This seems to be the only possibility. Our tRNA^{Phe} has pG–U on its 5′-end instead of pG–C. The end sequence pG–C–U–G in Euglena chls tRNA^{Phe} is paired with the tetranucleotide U–A–G–C, which does not exist in bean chls tRNA^{Phe}. In bean, U–G–G–C which replaces U–A–G–C, pairs perfectly with pG–U–C–G. Furthermore, in the 2/3 fragment, pU–C–G and pU–D–Gm–G–D–A–G could possibly occupy this position. The second corresponds to the characteristic sequence, containing the D and the invariant Gm_{18}–G_{19} and must therefore be situated in the D loop. Therefore pU–C–G must follow the 5′-end pGp.

Because of the extensive similarity between these two chloroplastic tRNAs^{Phe} further partial digestions were not performed.

4. Discussion

Bean chls tRNA^{Phe} is the first chloroplastic tRNA^{Phe} from higher plants sequenced so far. It is very similar to the first algal chloroplastic tRNA^{Phe} (*Euglena gracilis*) sequenced [1]. Five sequence differences and probably four differences in post-transcriptional modifications could exist between these two chloroplastic tRNAs^{Phe}. Like other known tRNAs^{Phe}, bean chls tRNA^{Phe} is 76 nucleotides long. It has only 3 nucleotides (4 in Euglena chls tRNA^{Phe}) which are different from the 48 nucleotides common to all known procaryotic tRNAs^{Phe} and 11 nucleotides (9 in Euglena chls tRNA^{Phe}) different from the 55 nucleotides common to all sequenced eucaryotic tRNAs^{Phe} disregarding the post-transcriptional modifications. This higher plant organellar tRNA^{Phe} seems to ressemble to a procaryotic tRNA^{Phe} even more than Euglena chls tRNA^{Phe}. In terms of modified nucleosides, these chls tRNAs^{Phe} look also more like procaryotic tRNAs. The m_2^2G found in bean chls tRNA^{Phe} digest [5] could be assimilated to an artefact spot because no m_2^2G could be identified in our present structural work. The position where m_2^2G is usually found is occupied by G in Euglena and A in bean, the latter one being characteristic of procaryotic tRNAs.

In addition both chls tRNAs^{Phe} differ by 24 nucleotides from their known counterparts in the cytoplasm of higher plants.

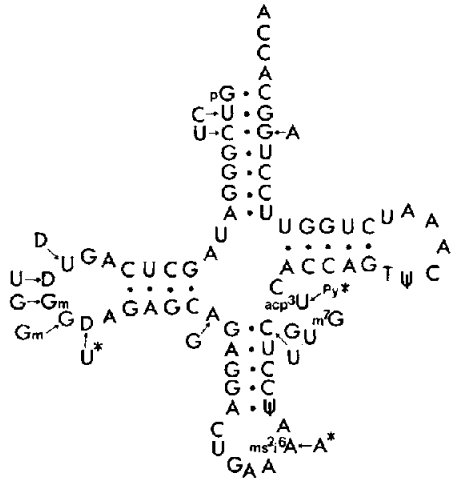

Fig. 2. Cloverleaf model of bean chls tRNA^{Phe}. Full arrows show the differences found between Euglena and bean chls tRNAs^{Phe}.

Acknowledgements

This work was carried out in the laboratories of Professors J. H. Weil and G. Dirheimer whose continuous support and stimulating criticism are gratefully acknowledged. We thank Dr Barnett for sending us a preprint of the article describing the sequence of Euglena chloroplast tRNA^{Phe} [1] prior to its publication. We are very grateful to Dr RajBhandary for sending us the fingerprints, and to Dr Hecker for giving us a sample of Euglena chloroplast tRNA^{Phe}, which have enabled us to make the sequence comparison described in this article. We thank also Dr Kleppe for having given us a sample of T_4 polynucleotide kinase and Dr H. J. Gross for useful advice at the beginning of our work on purification of that enzyme.

References

[1] Chang, S. H., Hecker, L., Silberklang, M., Brum, C. K., Barnett, W. E. and Raj Bhandary, U. L. (1976) Cell 9, 717–724.

[2] Dudock, B. S. and Katz, G. (1969) J. Biol. Chem. 244, 3069–3074.

[3] Everett, G. A. and Madison, J. T. (1976) Biochemistry 15, 1016–1021.
[4] Rafalski, A. J., Barciszewski, J., Gulewicz, K., Twardowski, T. and Keith, G. (1978) Acta Biochem. Polonica in press.
[5] Guillemaut, P., Martin, R. and Weil, J. H. (1976) FEBS Lett 63, 273–277.
[6] Simsek, M., Ziegenmeyer, J., Heckman, J. and RajBhandary, U. L. (1973) Proc. Natl. Acad. Sci. USA 70, 1041–1045.
[7] Richardson, C. C. (1965) Proc. Natl. Acad. Sci. USA 54, 158–165.
[8] Panet, A., Van de Sande, J. H., Loewen, P. C., Khorana, H. G., Raae, A. J., Lillehaug, J. R. and Kleppe, K. (1973) Biochemistry 12, 5045–5050.
[9] Glynn, I. M. and Chappell, J. B. (1964) Biochem. J. 90, 147–149.
[10] Fujimoto, M., Kuninaka, A. and Yoshino, H. (1974) Agr. Biol. Chem. 9, 1555–1561.
[11] Silberklang, M., Prochiantz, A., Haenni, A. L. and RajBhandary, U. L. (1977) Eur. J. Biochem. 72, 465–478.
[12] Sanger, F., Donelson, J. E., Carlson, A. R., Kössel, H. and Fischer, D. (1973) Proc. Natl. Acad. Sci. USA 70, 1209–1213.
[13] Simsek, M., Pétrissant, G. and RajBhandary, U. L. (1973) Proc. Natl. Acad. Sci. USA 70, 2600–2604.