5'- and 3'-terminal sequences of the chloroplast 16S and 23S ribosomal RNAs of *Euglena gracilis*

Murray N.Schnare, Gloria M.Yepiz-Plascencia¹, Donald W.Copertino¹, Richard B.Hallick¹ and Michael W.Gray*

Program in Evolutionary Biology, Canadian Institute for Advanced Research, Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada and ¹Department of Biochemistry, University of Arizona, Tucson, AZ 85721, USA

Submitted February 3, 1992

EMBL accession no. X12890

Prior to analysis of *Euglena gracilis* chloroplast 16S and 23S rRNAs, the sequence of an entire rRNA operon (*rrnC*) was completed, annotated in full, and deposited in the EMBL database (accession no. X12890, updated). To determine precisely the termini of the mature rRNAs, we directly sequenced the individual end-labeled species. 5'-Terminal and 3'-terminal sequences were determined by the enzymatic (1, 2) and chemical (3) methods, respectively. There were some minor bands in the sequencing ladders, most of which could be accounted for by cross-contamination with the other chloroplast rRNA species and/or cytoplasmic small subunit rRNA. Comparison of these RNA sequences with published DNA sequences allowed precise assignment of termini. Photographs of the relevant sequencing gels have been supplied to referees.

16S rRNA: Graf *et al.* (4) used T₁ oligonucleotide catalog data (5'-terminal oligonucleotide = pUG) and sequence alignments to tentatively identify the 5'-end of the 16S rRNA gene. Our data support this conclusion. Our data also confirm those of Steege *et al.* (5) in that the 16S rRNA ends with 3 C residues. However, the observed chemical hypersensitivity of the modified U residue at position 43 from the 3'-end is characteristic of 3-methyluridine (m³U) (6, 7) rather than the O²-methyluridine (Um) reported by Steege *et al.* (5). We have observed that Ump and m³Up are indistinguishable in the thin layer chromatography system used by Steege *et al.* (5). Our data also support the conclusion that there is only one N⁶,N⁶-dimethyladenosine residue (at position 23 from the 3'-end) in this region of *Euglena* chloroplast 16S rRNA (5, 8).

23S rRNA: When compared to the DNA sequence of the *Euglena* chloroplast 23S rRNA gene, the 5'-end of the rRNA (pUGAA-CA ...) maps to position 9 and the 3'-end (... CGAAAA_{OH})

maps to position 2882, according to the numbering of Yepiz-Plascencia *et al.* (9) (EMBL accession no. X13310; note that this sequence has recently been updated to incorporate the nucleotides AAG between positions 1733 and 1734 of ref. 9). Interestingly, placement of the termini at these positions precludes formation of the helix that has been modeled between the extreme 5'- and 3'-ends of other chloroplast as well as eubacterial 16S rRNAs (10).

ACKNOWLEDGEMENTS

This work was supported by operating grants from the Medical Research Council of Canada (MT-11212 to M.W.G.) and the U.S. National Institutes of Health (GM 35625 and 35665 to R.B.H), and by a fellowship from the Canadian Institute for Advanced Research (to M.W.G.).

REFERENCES

1. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) *Nucleic Acids Res.* **4**, 2527-2538.
2. Donis-Keller, H. (1980) *Nucleic Acids Res.* **8**, 3133-3142.
3. Peattie, D.A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1760-1764.
4. Graf, L., Roux, E., Stutz, E. and Kössel, H. (1982) *Nucleic Acids Res.* **10**, 6369-6381.
5. Steege, D.A., Graves, M.C. and Spremulli, L.L. (1982) *J. Biol. Chem.* **257**, 10430-10439.
6. Schnare, M.N. and Gray, M.W. (1982) *Nucleic Acids Res.* **10**, 3921-3932.
7. Schnare, M.N. (1984) PhD Thesis, Dalhousie University.
8. Van Buul, C.P.J.J., Hamersma, M., Visser, W. and Van Knippenberg, P.H. (1984) *Nucleic Acids Res.* **12**, 9205-9208.
9. Yepiz-Plascencia, G.M., Jenkins, M.E. and Hallick, R.B. (1988) *Nucleic Acids Res.* **16**, 9340.
10. Gutell, R.R., Schnare, M.N. and Gray, M.W. (1990) *Nucleic Acids Res.* **18** Suppl., 2319-2330.

* To whom correspondence should be addressed