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Nucleotide Sequence of Cytoplasmic 5S Ribosomal RNA from *Euglena gracilis*

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Summary. The nucleotide sequence of cytoplasmic 5S ribosomal RNA from *Euglena gracilis* has been determined to be: G-$\frac{A}{C}$-G-U-A-C-G-G-C-C-A-U-A-C-U-A-C-C-G-G-G-A-A-U-A-C-A-C-C-U-G-A-A-C-C-C-G-Ψ-U-C-G-A-U-U-U-C-A-G-A-A-G-U-U-A-A-G-C-C-G-G-G-U-U-A-G-G-C-C-C-A-G-U-U-A-G-U-A-C-U-G-A-G-U-G-G-G-C-G-A-C-C-A-C-U-U-G-G-G-A-A-C-A-C-U-G-G-G-U-G-C-U-G-U-A-C-G-C-U-Up. This RNA is 119 nucleotides long and the sequence of a probable tRNA-binding site is GAUU (position 41–44 from the 5'-terminus), which is the same as that of a trypanosoma species, *Crithidia fasciculata*. The *Euglena* 5S rRNA has a pseudouridine residue at position 38 and 3'-terminus is phosphorylated. The 5S rRNA sequence of *Euglena* resembles those of several other protozoa and higher animals rather than plants.

Key words: *Euglena gracilis* – 5S rRNA – RNA sequence – Protozoa – Phylogeny

Introduction

The phylogenetic position of *Euglena* has been unstable: it has been classified in some cases in plants or in other cases in protozoa. It has been also pointed out that *Euglena* has many biochemical characteristics of animal nature (Ragan and Chapman 1978). Since the 5S rRNA sequences are useful to establish the evolutionary relationships of organisms (Hori and Osawa 1979), we have sequenced the cytoplasmic 5S rRNA of *Euglena gracilis*. The *Euglena* sequence is closer to animal and several protozoan sequences than to plant sequences.

Materials and Methods

Cells of a plastid-less mutant of *Euglena gracilis* strain Z, CM500 were broken in a vibrator with glass beads in TMK buffer (50 mM Tris-HCl, pH 7.6, 10 mM Mg-acetate, 25 mM KCl). After removing the cell debris and mitochondria, 80S ribosomes were pelleted by centrifugation. The ribosomes so obtained were dissolved in a buffer containing sodium dodecyl sulfate (SDS) (0.5% SDS, 20 mM Tris-HCl, pH 7.4, 30 mM $MgCl_2$, 50 mM NaCl), and shaken with 90% phenol three times to release rRNAs to the water phase. For the isolation of 5S rRNA, rRNAs that had been precipitated with 3 volumes of ethanol were electrophoresed on a 4% polyacrylamide gel. The crude 5S rRNA recovered from the gel was electrophoresed again on a 15% polyacrylamide gel to obtain 5S rRNA having a purity sufficient for sequencing.

The sequencing procedures used were those described by Peattie (1979) and Kuchino et al. (1979).

Results

The cytoplasmic 5S rRNA sequence of *Euglena gracilis* which has been established in this study is shown in Fig. 1, together with the sequences of *Drosophila*, *Crithidia* and wheat 5S rRNAs for comparison. The ^{32}P-labelling of the 3'-terminus was done with [5'-^{32}P]pCp after a pretreatment of the 5S rRNA with alkaline phosphatase, because the 3'-terminus had been already phosphorylated. The sequences of 110 nucleotides from the 3'-terminus were established by the method of Peattie (1979) using 3'-^{32}P-labelled RNA. To sequence the 5'-terminal region and to detect minor bases, if any, the 5S rRNA was hydrolyzed by formamide, followed by labelling with [γ-^{32}P]ATP of the 5'-end of the products.

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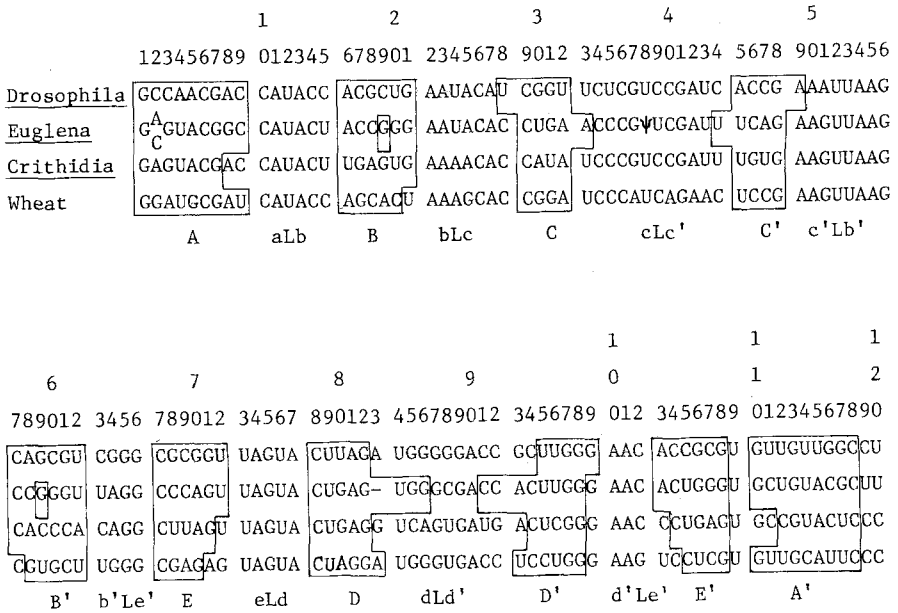

Fig. 1. Comparison of 5S rRNA sequences of *Euglena gracilis*, *Drosophila melanogaster*, *Crithidia fasciculata* and wheat. The squared off-sequences correspond to the base-paired regions in the secondary structures (A/A', B/B', C/C', D/D' and E/E' in the figure). aLb, bLc, etc. are symbols for loop regions (e.g., aLb is the loop region between A and B)

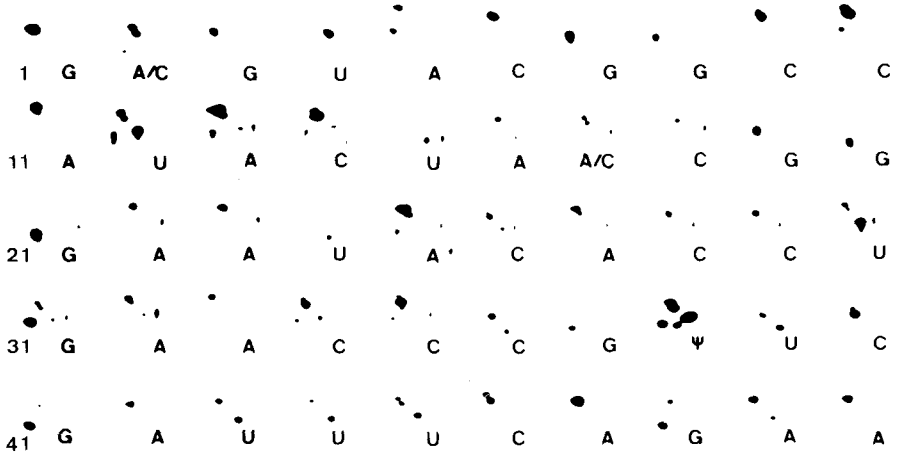

Fig. 2. Analysis of the 5'-terminal nucleotides of the fragments of *Euglena gracilis* 5S rRNA by two-dimensional thin-layer chromatography

After polyacrylamide gel electrophoresis of the hydrolysates, the base of the 5'-end of each fragment band was identified by chromatography on a TLC plate according to Kuchino et al. (1979). Fifty nucleotides from the 5'-terminus were determined in this way (Fig. 2). Both A and C were detected at position 2. This probably represents the heterogeneity of the *Euglena* 5S rRNA. Two spots A and C were also detected at position 17, but the A was probably due to a contamination of the residue at position 16, since the chemical sequencing from the 3'-terminus showed that the 17th residue was C. Pseudouridine was detected at position 38.

To analyze the 5'-terminal nucleotide, the 5'-terminus-labelled RNA was digested by nuclease P_1 and chromatographed on a TLC plate. Only G was detected as the 5'-terminal base. The 3'-terminus was determined to be U (U:G = 96:4) by hydrolyzing the 3'-terminus labelled RNA followed by the TLC identification of the radioactive nucleotides.

Discussion

Fig. 3 shows a secondary structure of the *Euglena gracilis* cytoplasmic 5S rRNA constructed from its primary sequence by the method previously described (Hori and Osawa 1979; Osawa and Hori 1979). In our 5S rRNA secondary structure model, the eukaryotic stem D-D' usually consists of 5 or 6 base pairs, and the length of the loop dLd' is rather variable ranging between 9 and

Table 1. Sequence homology of various eukaryotic 5S rRNAs (%)

	HUM	DME	EGR	CFA	TTH	ACA	DDI	SPO	SCE	CCO	CRE
DME	78										
EGR	74	73									
CFA	61	63	72								
TTH	64	73	70	67							
ACA	67	68	68	68	68						
DDI	63	58	68	65	60	66					
SPO	72	68	69	68	68	69	70				
SCE	61	56	58	58	57	64	56	69			
CCO	65	68	61	55	70	64	52	60	54		
CRE	56	47	54	54	57	58	46	52	53	50	
WHE	66	65	61	58	68	65	55	61	60	64	60

The sequences of human (HUM), *Drosophila melanogaster* (DME), *Crithidia fasciculata* (CFA) and *Saccharomyces cerevisiae* (SCE) were cited from Erdmann (1981). That of *Tetrahymena thermophila* (TTH) from our unpublished data and Erdmann (1981), *Acanthamoeba castellanii* (ACA) from MacKay and Doolittle (1981), *Dictyostelium discoideum* (DDI) from Hori et al. (1981), *Schizosaccharomyces pombe* (SPO) from Komiya et al. (1981), *Crypthecodinium cohnii* (CCO) from Hinnebusch et al. (1981), *Chlamydomonas reinhardii* (CRE) from Darlix and Rochaix (1981), and wheat (WHE) from Mackay et al. (1980)

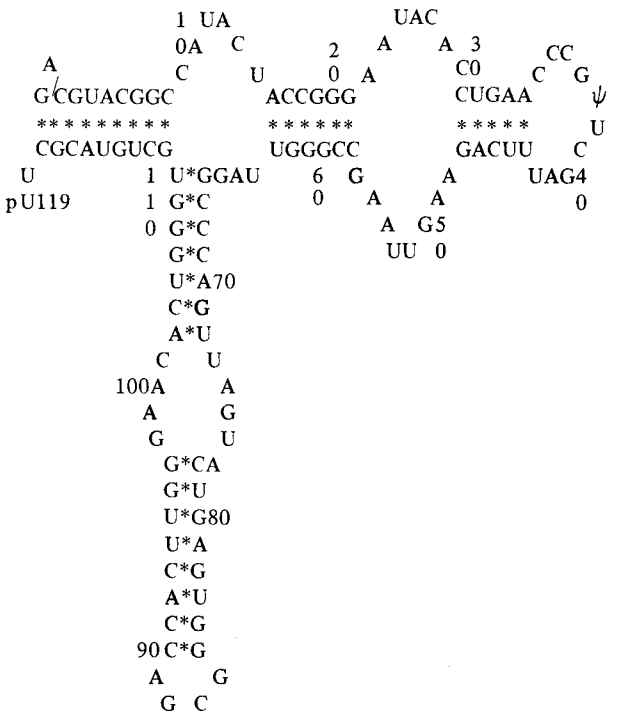

Fig. 3. Secondary structure of *Euglena gracilis* cytoplasmic 5S rRNA

12 nucleotides long (Hori et al. 1980; Luehrsen and Fox 1981). However, the *Euglena* 5S rRNA may be exceptional or extreme in this respect; the stem D-D' consists of 8 base pairs and the loop dLd' is only 4 nucleotides long. These features apparently approach those of eubacterial 5S rRNAs. On the other hand, the presence of a base-paired region, stem E-E', is a common feature in the eukaryotic 5S rRNAs (Hori et al. 1980; Luehrsen and Fox 1981), and the *Euglena* 5S rRNA does have it.

The sequence GA$^{U}_{A}$C in the cLc' region, a probable tRNA-binding site, has been found in most of the 5S rRNAs including those of *Tetrahymena* and *Acanthamoeba*. In *Euglena*, the corresponding sequence is GAUU, which has so far been found only in 5S rRNA of a trypanosoma species, *Crithidia fasciculata*. This would suggest some evolutionary relationship of *Euglena* to the trypanosoma.

Table 1 is a similarity matrix of 5S rRNA sequences for several representative eukaryotes. The sequence similarity of *Euglena* with several protozoa (*Tetrahymena thermophila*, *Crithidia fasciculata* and *Acanthamoeba castellanii*), with a part of fungi (*Dictyostelium discoideum* and *Schizosaccharomyces pombe*) and with animals, is almost comparable (68–74% similarity), while with wheat, with *Chlamydomonas reinhardii*, with *Saccharomyces cerevisiae* and with a dinoflagellate, *Crypthecodinium cohnii*, the sequence similarity is considerably less (54–61% similarity). It has been also reported that the nucleotide sequence of cytoplasmic tRNA^{phe} from *Euglena gracilis* resembles that from beef much more than that from wheat (Chang et al. 1981). These facts suggest that *Euglena* is phylogenically more related to animals than to plants; the classification of *Euglena* as a member of plants is indeed doubtful, even though *Euglena* cells normally contain chloroplasts.

Note Added in Proof

At the time we submitted our manuscript, Delihas et al. (1981) published the 5S rRNA sequence of *Euglena glacilis* which was similar but not identical to ours.

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