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The 5S ribosomal RNA of Euglena gracilis cytoplasmic ribosomes is closely homologous to the 5S RNA of the trypanosomatid protozoa

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ABSTRACT

The complete nucleotide sequence of the major species of cytoplasmic 5S ribosomal RNA of Euglena gracilis has been determined. The sequence is: 5' GGCGUACGGCCAUACUACCGGGAAUACACCUGAACCCGUUCGAUUUCAGAAGUUAAGCCUGGUCAGG CCCAGUUAGUACUGAGGUGGGCGACCACUUGGGAACACUGGGUGCUGUACGCUU$_{OH}$ 3'

This sequence can be fitted to the secondary structural models recently proposed for eukaryotic 5S ribosomal RNAs (1,2). Several properties of the Euglena 5S RNA reveal a close phylogenetic relationship between this organism and the protozoa. Large stretches of nucleotide sequences in predominantly single-stranded regions of the RNA are homologous to that of the trypanosomatid protozoan Crithidia fasiculata. There is less homology when compared to the RNAs of the green alga Chlorella or to the RNAs of the higher plants. The sequence AGAAC near position 40 that is common to plant 5S RNAs is CGAUU in both Euglena and Crithidia. The Euglena 5S RNA has secondary structural features at positions 79-99 similar to that of the protozoa and different from that of the plants. The conclusions drawn from comparative studies of cytochrome c structures which indicate a close phylogenetic relatedness between Euglena and the trypanosomatid protozoa are supported by the comparative data with 5S ribosomal RNAs.

INTRODUCTION

The unicellular photosynthetic and non-photosynthetic eukaryotes are a heterogeneous group of organisms and their phylogenic relationships have not been clearly elucidated. The photosynthetic protist Euglena has been difficult to classify because of its diverse properties. Although tentatively classified as an alga because of its photosynthetic properties (3), a comparison of cytochrome c structures closely links this organism to the trypanosomatid protozoa (4). A recent report on the structure of the cytoplasmic phenylalanine tRNA of Euglena shows a high homology of this tRNA to the RNA of mammalian cells (5). On the other hand, Euglena appears to have a pathway for lysine biosynthesis that is similar to that of the

higher fungi (6).

The 5S ribosomal RNAs have been used to establish phylogenetic relationships between different organisms (2,7). We report here the nucleotide sequence of the 5S RNA from cytoplasmic ribosomes of Euglena. This RNA is more homologous to the 5S RNA of the trypanosomatid protozoan Crithidia than to the 5S RNAs of the plants and green algae. These data support the classification of Euglena as a protozoan.

EXPERIMENTAL PROCEDURE

Isolation and Nucleotide Sequence Analysis of the 5S RNA from Euglena gracilis Cytoplasmic Ribosomes. Euglena gracilis Klebs, Z strain, Pringshein, was grown heterotrophically in the presence of white light at 26°C on pH 3.5 organotropic media (8) or on pH 5 glucose-based media (9). Cells were grown to a concentration of 2-5x10^6 cells/ml in a volume of 24 liters. Upon harvesting, cells were disrupted in a French Pressure Cell after the method of Lyman and Traverse (8) in a medium containing 50mM Tris-HCl pH 7.8, 3mM $Mg(oAc)_2$, 75mM NH_4Cl, 5mM β-mercaptoethanol, 160mM sucrose. Centrifugation at 30,000 rpm for 10 minutes removed chloroplasts and membranes. Ribosomes were isolated by centrifugation at 95,500xg for 3 hours. Total ribosomal RNA was extracted by phenol. Low molecular weight RNA was separated from high molecular weight RNA by resuspending the precipitated total RNA in 0.1M K(oAc) pH 6.5 and centrifuging at 10,000 rpm for 10 minutes. The 5S RNA fraction was partially purified by Sephadex G-200 column chromatography of the low molecular weight RNA supernatant. Final purification was by 12% polyacrylamide gel electrophoresis in 7M urea (10).

The nucleotide sequence of the 5S ribosomal RNA of Euglena was determined with 3 overlapping sequencing methods (11).

RESULTS

One major species of 5S RNA and several minor RNAs were isolated from Euglena gracilis cytoplasmic ribosomes. These RNAs migrate close to marker E. coli 5S RNA in 7M urea - 12% polyacrylamide gels; they can be labeled in vitro at the 3' end with [5'-^{32}P] pC_p and T4 RNA ligase. Mobility shift analyses (12) show that these RNAs differ in nucleotide sequence at the 3' end. The structure of the major species of 5S ribosomal RNA was determined completely and is reported here.

Partial formamide digestion and internal 5' labeling (13), RNA sequencing gels (10) and mobility shift analyses (12) were used to provide overlapping and confirming data on the nucleotide sequence of the 5S RNA of Euglena cytoplasmic ribosomes. The sequence and a secondary structural model for the 5S RNA are given in Fig. 1. For comparison, the 5S RNA structures of Crithidia fasiculata (protozoan) (14), Chlorella pyrenoidosa (green alga) (1) and Spinacia oleracia (spinach) (11) are also shown. The lines drawn around the sequences in Crithidia represent large stretches of

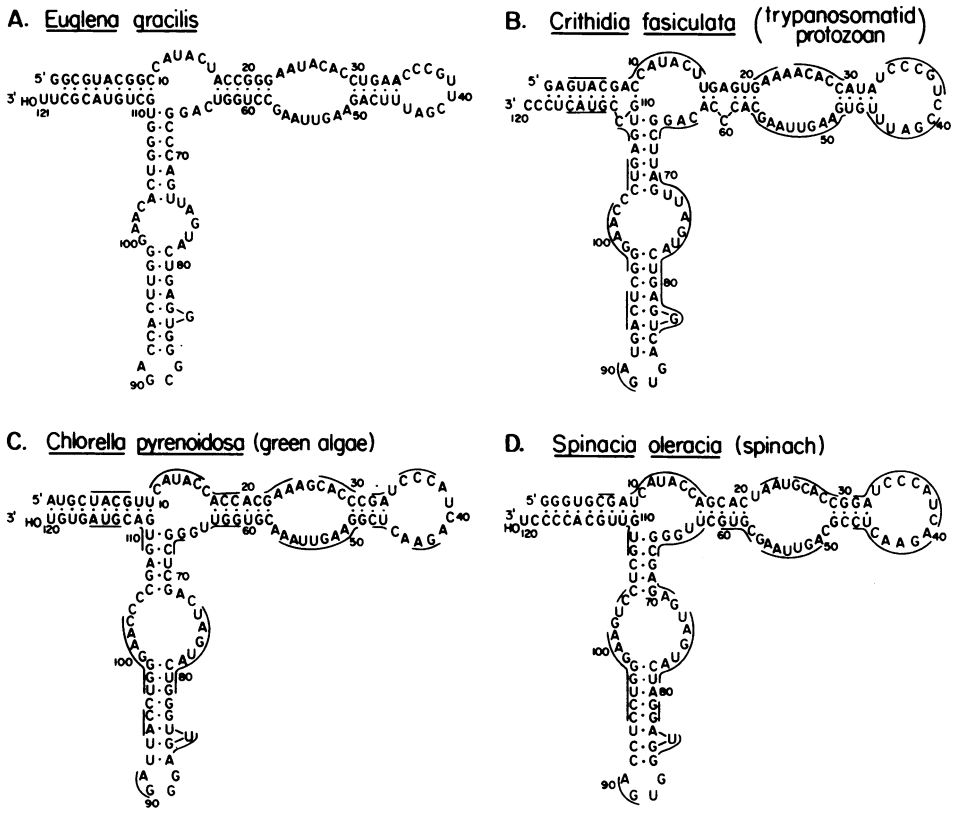

Fig. 1. 5S ribosomal RNA sequences and secondary structural models. The lines drawn around the sequences of Crithidia (B) and Chlorella (C) denote large stretches of homology between these organisms and Euglena. The lines drawn around the 5S RNA structure of spinach (D) denote stretches of homology between spinach and Chlorella.

homology between Euglena and Crithidia. Although we have no secondary structural data for Euglena, secondary structures are drawn according to the model of Luehrsen and Fox (1) for eukaryotic 5S RNAs, but include added base-pairing between positions 79-99 that has been proposed for this region by KÜntzel et al (2). These models are based on comparative analysis. Metazoan, protozoan and plant 5S RNAs appear to have characteristic secondary structural properties in this region. Thus, the structure of this segment of 5S RNA is of major importance in phylogenetic studies.

DISCUSSION

The nucleotide sequence of the 5S RNA from Euglena cytoplasmic ribosomes has primary and possible secondary structural features common to other eukaryotic 5S RNAs (Fig. 1). One major difference is the 5 base-pairs and a 10 membered single-stranded loop that can be formed for the Euglena 5S ribosomal RNA within positions 30-49 compared to 4 base-pairs and a 12 residue loop for other eukaryotic 5S RNAs.

The structure of Euglena 5S ribosomal RNA is similar to that of the animal 5S RNAs but especially to that of the trypanosomatid protozoan Crithidia. 1. Large stretches in nucleotide sequences are homologous between Euglena and Crithidia (Fig. 1). 2. The percent overall homology between 5S RNAs of Euglena and Crithidia is 73% and between Euglena and Human KB cell RNAs it is 74%; however the homology is less between Euglena and other organisms (Table 1). In single-stranded regions, the homology between Euglena and Crithidia 5S RNAs is as high as 94%. 3. The sequence C_{41} GAUU in Euglena 5S RNA is identical to that in Crithidia 5S RNA and different from sequences characteristic to plants (AGAAC) or animals and fungi (PyGAUC) (15). However, Tetrahymena (16), Chlamydomonas (17), Neurospora and Aspergillus (18) appear to have their own sequences in this region. 4. The secondary structure proposed for positions 79-99 of the 5S RNA from Euglena (Fig. 1) is characteristic for that of the RNAs of protozoa and fungi (2). A guanine residue is looped-out at position 83 with 5 base pairs on one side and 3 base-pairs on the other adjacent to this looped-out position. Dictyostelium (19), Chlamydomonas (17), Torulopsis (20), Tetrahymena (16), and Aspergillus (18) all have this characteristic structure. The plants and Chlorella have a looped-out uracil residue with 6 and 2 base-pairs adjacent to the looped-out position (Fig. 1). The metazoans have 2 looped-out positions, cytosine and adenine residues, which are opposite each other and have 5 and 3 base-pairs adjacent to the looped-

Table 1

Sequence Homology Between *Euglena* 5S RNA and 5S RNAs of Other Organisms

Source of 5S RNA	% Overall Homology to *Euglena* 5S RNA	% Homology in Single Stranded Regions to *Euglena* 5S RNA
Crithidia	73	94
Human KB Cell	74	78
Torulopsis	59	81
Chlorella	64	77
Spinach	64	81

out positions (2). Thus, the *Euglena* 5S RNA from cytoplasmic ribosomes has primary and secondary structural features different from the plants but close to that of the protozoa.

Other properties of *Euglena* also indicate a relatedness to the protozoa of other animals. A comparison of cytochrome c structures show a close relationship between *Euglena* and *Crithidia* (4). The flagellar structures of *Euglena* and the trypanosomes are similar (21). A recent report on phenylalanine tRNA nucleotide sequences shows a close homology between *Euglena* and mammalian cell cytoplasmic phenylalanine tRNAs and much less homology between *Euglena* and plant phenylalanine tRNAs (5). Thus, much of the information on non-chloroplast molecular structures indicates that *Euglena* is more closely related to protozoa or mammalian cells than green plants.

Euglena may also differ from the higher plants in the nature and origin of its chloroplasts. It is of interest to note that the chloroplast 5S

ribosomal DNA sequences of Euglena (Dodd, J., Karabin, J. and Hallick, R. personal communication) differs from the chloroplast 5S RNAs of plants (22-24). The sequence homology between the chloroplast 5S RNAs of Lemna (duckweed) (23) and of Spinacea oleracia (spinach) (22) is 99%, whereas the sequence homology between the spinach chloroplast 5S RNA and the two different 5S ribosomal DNA genes of Euglena is 52-53%. This may indicate a different origin of the Euglena chloroplast compared to that of green plant chloroplasts.

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