The nucleotide sequence of 5 S ribosomal RNA from a protozoan species *Chilomonas paramecium* belonging to the class Phytomastigophorea

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1. INTRODUCTION

In the protozoan class Phytomastigophorea, the 5 S rRNA sequences from three species, *Crypthecodinium cohnii* (order Dinoflagellida), *Euglena gracilis* (order Euglenida) and *Chlamydomonas reinhardii* (order Volvocida), are known. These 5 S rRNAs do not show any striking similarity to each other, suggesting that these orders, although classified in the same class Phytomastigophorea, are only remotely related to each other. Here, we have determined the sequence of 5 S rRNA of another Phytomastigophorea species, *Chilomonas paramecium* (order Cryptomonadida), and compared it to those of the other species of the same class as well as of other eukaryotes to deduce the phylogenic position of *Chilomonus*. The *Chilomonas* sequence shows a fairly low but almost equal similarity to the sequences of the other Phytomastigophorea species and also to those of other protozoa, plants, fungi and animals.

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2. EXPERIMENTAL

The 5 S rRNA of *C. paramecium* was isolated by phenol method from the whole cells and purified by electrophoresis on a 15% polyacrylamide gel as in [1]. The 3'- or 5'-terminal base analysis was done as in [2]. The nucleotide sequence was determined by the chemical method in [3] and the enzymatic method in [4].

3. RESULTS

3.1. *3'- and 5'-terminal base analyses*

When the 5 S r[3'-^{32}P]RNA was digested completely with RNase T_2 followed by the analysis of radioactive base by thin-layer chromatography [2], only Cp* (* = radioactive) was detected. When the 5 S r[5'-^{32}P]RNA was digested with nuclease P_1, only p*U was observed. These results indicated that the 3'-terminal base was C and the 5'-terminal was U.

3.2. *Sequence analysis*

The sequence of 120 nucleotides from the 3'-terminus was determined by the chemical method [3] and confirmed by the enzymatic method [4] using [3'-^{32}P]RNA. The sequence of the 5'-terminal region was also confirmed by the same enzy-

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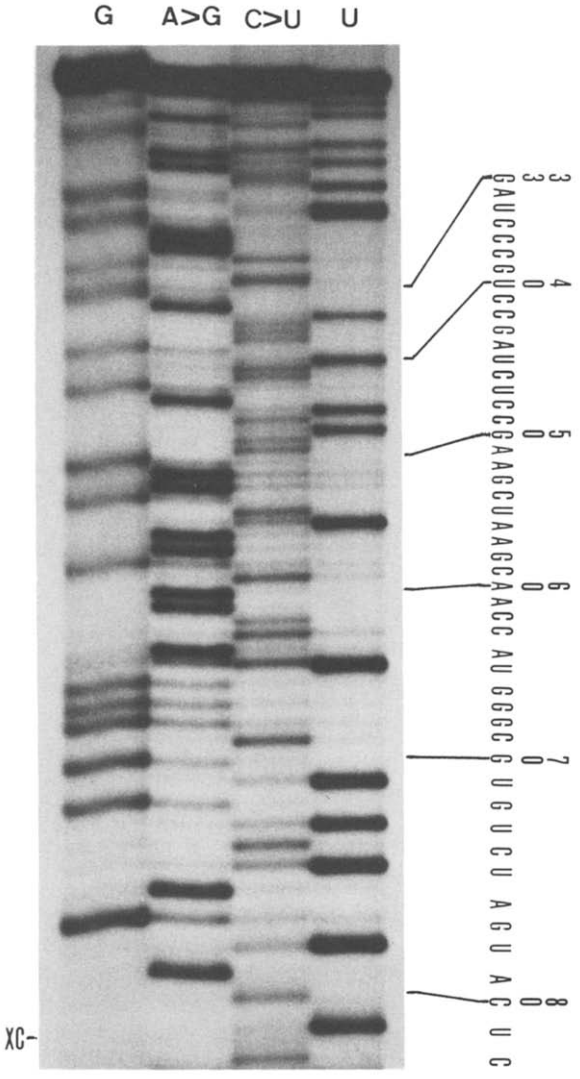

Fig. 1. Autoradiogram of 5 S r[3′-^{32}P]RNA of *Chilomonas paramecium*. The partial chemical digests [3] were fractionated on 12% polyacrylamide gel in 7 M urea, 0.1 M Tris–borate (pH 8.3) and 1 mM EDTA at 1500 V for 7 h. The residue number corresponds to that of the alignment in fig. 2. *Abbreviation:* XC, marker dye: xylene cyanol.

matic method using [5′-^{32}P]RNA. An autoradiogram of the sequencing gel obtained by the chemical method is shown in fig. 1, where nucleotides in positions 33–82 are readable. The *C. paramecium* 5 S rRNA is 121 nucleotides long and its sequence is shown in fig. 2 with the sequences of the other three Phytomastigophorea species, *C. cohnii* [5], *E. gracilis* [6] and *C. reinhardii* [7] for comparison.

4. DISCUSSION

The 5 S rRNA sequences of the four species can be easily aligned with insertions of a few gaps (fig. 2). As already pointed out [8,9], the sequences of the loop regions in the secondary structure are more conserved (57%; No. of identical sites in all the four sequences/No. of sites compared) than the base-paired regions (31–33% identity) among these four Phytomastigophorea species.

We have proposed a model for the eukaryotic 5 S rRNA using the sequence of the *Dictyostelium discoideum* 5 S rRNA [10], in which the region dLd′ (see fig. 2 and fig. 3) forms a fairly large loop. Since then, considerable numbers of the eukaryotic sequences have become available. The examinations of these sequences suggest that the number of the basepairs in the D–D′ stem can increase from 5–8 to have a more stable structure, if a bulge of one G (the protozoa-fungi type), a bulge of one U (the plants type) or one A/C mismatch (multicellular animals type) is allowed (see fig. 3, and fig. 3 in [1]; see also [9,11]). Also, the B–B′ stem can be longer (8 instead of 6) with a bulge of one base in all the 5 S rRNAs (see also [11,12]). The secondary structure of the protozoa-fungi type as described above can be constructed with the 5 S rRNAs from *Chilomonas* and other Phytomastigophorea species, with exception of the *Euglena* 5 S rRNA where the bulge in the D–D′ stem does not exist. In the *Chilomonas* 5 S rRNA, two bases from the 5′-terminus cannot base-pair.

Table 1 shows a similarity matrix of the 5 S rRNA sequences from eukaryotes. The similarity of *Chilomonas* to other Phytomastigophorea species is only from 57–64% with the average of 61%. These values are not very much different from the similarities between *Chilomonas* and other protozoa (61–66%), plants (60%), fungi (61%) or animals (63%), suggesting that, although *Crypthecodinium, Euglena, Chlamydomonas* and *Chilomonas* have been included in the same class, Phytomastigophorea, they are no more related to each other than to other eukaryotes, and the divergence of these protozoa initiated at about the same period as that of other principal eukaryotic groups.

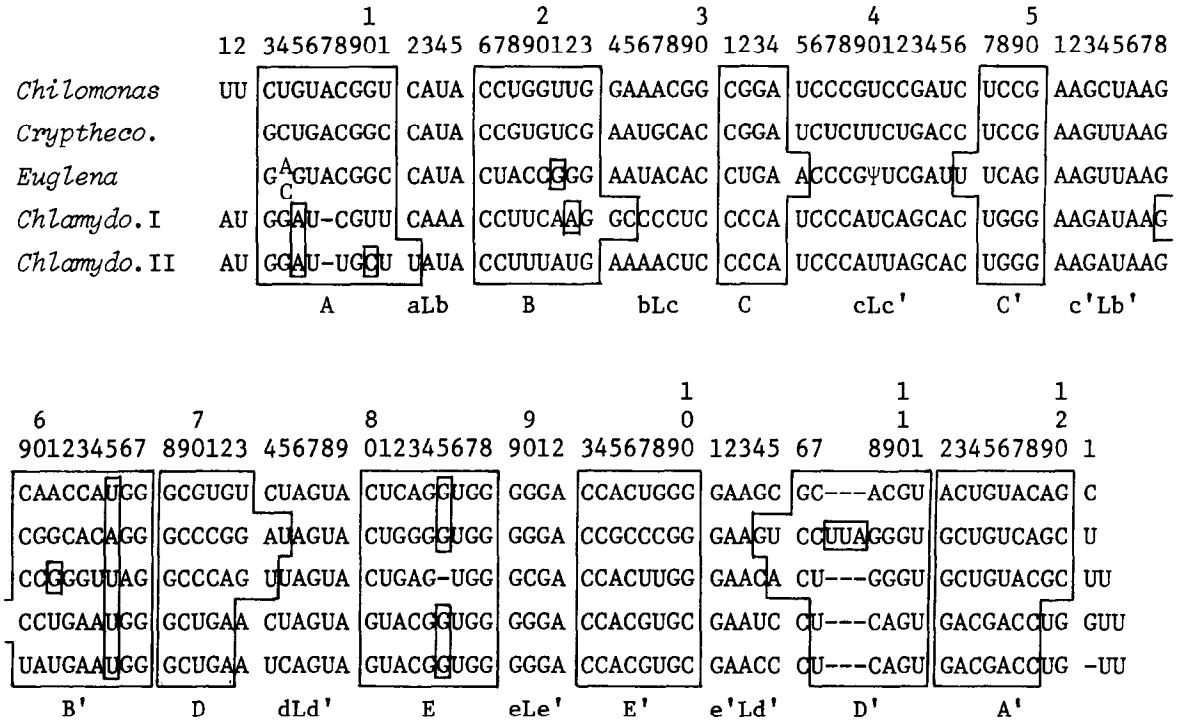

Fig. 2. Comparison of 5 S rRNA sequences of *Chilomonas paramecium, Crypthecodinium cohnii, Euglena gracilis* and *Chlamydomonas reinhardii*. The squared-off sequences correspond to the base-paired regions in the secondary structures (A,A', B,B', in the lowest line). Symbols, aLb, bLc, are for loop regions (see [10,13]).

Table 1

Similarity matrix of 5 S rRNA sequences of eukaryotes (%)

	CPA	CCO	EGR	CRE	CFA	ACA	CIL	PLA	FUN	ANI
CPA		64	61	57	66	61	63	60	61	63
CCO	64		68	52	58	70	74	64	63	71
EGR	61	68		54	72	68	68	60	60	73
CRE[b]	57	52	54		56	57	56	57	52	53
CFA	66	58	72	56		68	64	54	60	61
ACA	61	70	68	57	68		69	61	66	66
CIL[a]	63	74	68	56	64	69		66	60	66
PLA[a]	60	65	60	57	55	62	66		57	63
FUN[a]	61	63	60	52	60	66	60	57		61
ANI[a]	63	71	73	53	61	66	66	62	61	

[a] The mean similarity values calculated from the sequences of 3 ciliated protozoa (CIL), 10 plants (PLA), 12 fungi (FUN) and 37 animals (ANI)

[b] The mean similarity values of 2 heterogenous sequences (see fig. 1)

Abbreviations: CPA, *Chilomonas paramecium*; CCO, *Crypthecodinium cohnii*; EGR, *Euglena gracilis*; CRE, *Chlamydomonas reinhardii*; CFA, *Crithidia fasciculata*; ACA, *Acanthamoeba castellanii*

For the sources of the sequences, see [1,8]

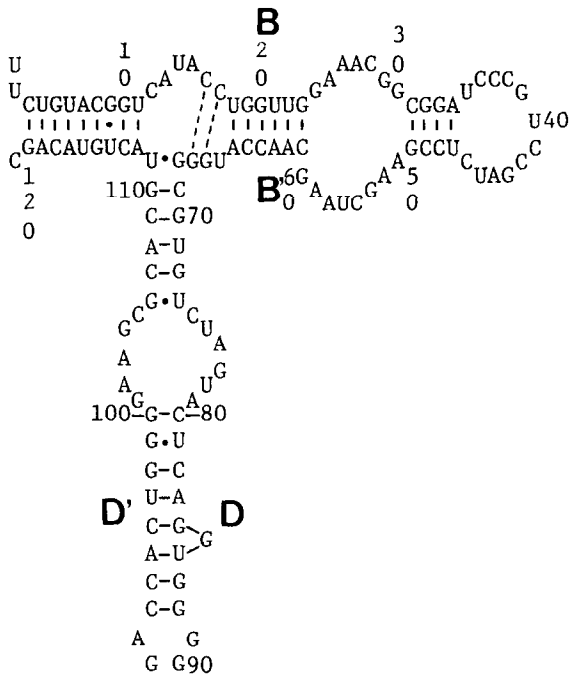

Fig. 3. Secondary structure model of *Chilomonas paramecium* 5 S rRNA.

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