Volume 10 Number 14 1982 Nucleic Acids Research

The nucleotide sequences of 5S rRNAs from three ciliated protozoa

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Received 19 May 1982; Accepted 22 June 1982

ABSTRACT

The nucleotide sequences of 5S rRNAs from three ciliated protozoa, *Paramecium tetraurelia*, *Tetrahymena thermophila* and *Blepharisma japonicum* have been determined. All of them are 120 nucleotides long and the sequence of probable tRNA binding site of position 41-44 is GAAC which is characteristic of the plant 5S rRNAs. The sequence similarity percents are 87 % (*Paramecium/Tetrahymena*), 86 % (*Paramecium/Blepharisma*) and 79 % (*Tetrahymena/Blepharisma*), suggesting a close relationship of these three ciliates.

INTRODUCTION

Ciliated protozoa (Ciliophora) have been morphologically divided into three classes, "the most primitive" Class I ciliates, "more advanced" Class II ciliates and "the most advanced" Class III ciliates (1). Since the 5S rRNA sequences are useful for establishing the evolutionary relationship of organisms (2), we have sequenced 5S rRNAs from three species of ciliated protozoa, *Paramecium* (Class II), *Tetrahymena* (Class II) and *Blepharisma* (Class III) (i) to see if the relationships morphologically deduced agree with those from the sequences, though the Class I species have not been available in this study, and (ii) to deduce the phylogenic position of the ciliates relative to other organisms.

MATERIALS AND METHODS

The 5S rRNA of *Paramecium tetraurelia* (mating type VIII) or *Blepharisma japonicum* R13 was prepared by the phenol method from 80S ribosomes and purified by electrophoresis on a 15 % Polyacrylamide gel as described before (3). The 5S rRNA of *Tetrahymena thermophila* (mating type IV) was directly prepared by the phenol-cresol method from the whole cells and purified by electrophoresis on a 10 % polyacrylamide gel.

The sequences were determined mainly by the method of Peattie (4) using

0305-1048/82/1014-4409$2.00/0

3'-^{32}P-labelled 5S rRNAs. Some parts of the sequences derived from the above analyses were confirmed by the enzymatic method (5). The sequencing of the 5'-terminal regions and the detection of minor bases in *Tetrahymena* and *Blepharisma* 5S rRNAs were done according to Kuchino et al. (6).

RESULTS

(1) 5'- and 3'-end analyses

The 5'- or 3'-end labelled 5S rRNAs from the three species were digested completely by nuclease P_1 or RNase T_2 and chromatographed on a cellulose TLC plate (3). The autoradiograms showed that the 5'-end nucleotide of all the three species was G, and that the 3'-end of *Paramecium* was C, that of *Tetrahymena* was U, and that of *Blepharisma* was A.

(2) Sequencing of 5S rRNAs

The sequences of 119 (*Paramecium*) or 110 nucleotides (*Tetrahymena* and *Blepharisma*) from the 3'-end of the 5S rRNAs were determined by the chemical degradation of [3'-^{32}P] RNAs followed by the electrophoresis (4). The sequences so resulted were confirmed by the enzymatic method as described by Donis-Keller (5). Some parts of the sequence were also confirmed by electrophoresis at 70°C on a hot plate (7). The sequence of position 1-10 from the 5'-end of *Tetrahymena* or *Blepharisma* was effectively established by the method of Kuchino et al. (6) with the confirmation of the sequences of

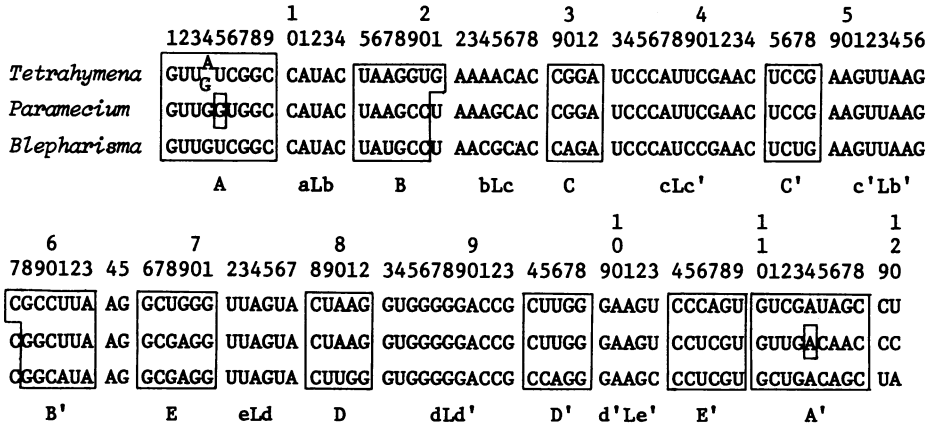

Figure 1. Sequence alignment of 5S rRNAs of *Paramecium tetraurelia*, *Tetrahymena thermophila* and *Blepharisma japonicum*. The squared-off sequences correspond to the base-paired regions in the secondary structure (A,A', B,B', etc. in the lowest line). aLb, bLc, etc. are symbols for loop regions (e.g., aLb is the loop region between A and B; for these symbols, see refs. 2, 8).

certain other regions. No minor bases were detected in these 5S rRNAs. Figure 1 shows the primary sequences of 5S rRNAs from the three species deduced from the above analyses. All these 5S rRNAs were 120 nucleotides long and the sequences were aligned without gaps.

DISCUSSION

The secondary structure of 5S rRNAs from the three ciliates (not shown) is essentially the same as that previously proposed for eukaryotic 5S rRNAs (8). The sequences of the loop regions are highly conserved [90 % similarity (52/58)] while those of base-paired regions are not [63 % similarity (39/62)] among these three species.

The similarity matrix of the 5S rRNA sequences (Table 1) indicates that *Paramecium*, *Tatrahymena* and *Blepharisma* are closely related to each other. The similarity percents of *Paramecium*/*Tetrahymena*, *Paramecium*/*Blepharisma*, and *Tetrahymena*/*Blepharisma* are 87 %, 86 % and 79 %, respectively. Thus, *Tetrahymena* (Class II) seems closer to *Paramecium* (Class II) than to *Blepharisma* (Class III), supporting the relationship derived from the morphological studies (1). The similarity matrix further suggests that the ciliates are more related to other protozoa, e.g., *Acanthamoeba* (68–72 %) and *Euglena* (66–69 %), than to Ascomycetes (57–59 %). Since these ciliates show almost equal similarities to plants (63–70 %) and to animals (64–68 %),

Table 1. Similarity matrix of 5S rRNA sequences of representative eukaryotes (%).

	PTE	TTH	BJA	ACA	EGR	ASC	PLA*
ANI*	64	66	68	66	72	59	63
PTE		87	86	68	66	58	70
TTH			79	68	69	57	63
BJA				72	66	59	68
ACA**					68	64	61
EGR**						57	59
ASC*							56

*Mean values of 17 higher animals (ANI), 8 Ascomycetes species (ASC) and 8 higher plants (PLA) were calculated from the sequences in ref. 11.
**The sequence of *Acanthamoeba castellanii* (ACA) was cited from ref. 12. That of *Euglena gracilis* (EGR) from ref. 3. Abbreviations: PTE, *Paramecium tetraurelia*; TTH, *Tetrahymena thermophila*; BJA, *Blepharisma japonicum*.

they might have diverged at about the same time. However, the sequence of a probable tRNA binding site (position 41-44; ref. 9) in the 5S rRNAs of these ciliates is GAAC which is characteristic of the plant 5S rRNAs. This would suggest a closer relationship between the ciliates and plants than between the ciliates and animals.

During the course of this study, Luehrsen et al. (10) published the sequence of *Tetrahymena* 5S rRNA, which agrees with ours except that they recognized U/C heterogeneity at position 2.

ACKNOWLEDGEMENTS

We thank Professor R. Katashima, Dr. T. Kosaka and Dr. T. Matsuoka, Department of Zoology, Hiroshima University, Japan, for the gift of the strains used in this study and valuable advices. This work was supported by the Grant-in-Aids for Scientific Research of Japan (Nos. 56480377, 56570178 and 56108012).

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