Volume 15 Number 19 1987 Nucleic Acids Research

Unusual ribosomal RNA of the intestinal parasite *Giardia lamblia*

Thomas D.Edlind[1] and Prasanta R.Chakraborty[1,2]

[1]Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, PA 19129 and [2]Division of Biochemical Parasitology, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065, USA

Received May 14, 1987; Revised and Accepted September 2, 1987

ABSTRACT

The anaerobic protozoan Giardia lamblia is a common intestinal parasite in humans, but is poorly defined at molecular and phylogenetic levels. We report here a structural characterization of the ribosomal RNA (rRNA) and rRNA genes of G. lamblia. Gel electrophoresis under native or non-denaturing conditions identified two high molecular weight rRNA species corresponding to the 16-18S and 23-28S rRNAs. Surprisingly, both species (1300 and 2300 nucleotides long, respectively) were considerably shorter than their counterparts from other protozoa (typically 1800 and 3400 nucleotides), and from bacteria as well (typically 1540 and 2900 nucleotides long). Denaturing polyacrylamide gel electrophoresis identified a major low molecular RNA of 127 nucleotides and several minor species, but no molecules with the typical lengths of 5.8S (160 nucleotides) and 5S (120 nucleotides) rRNA. The G. lamblia 1300, 2300, and 127 nucleotide RNAs are encoded within a 5.6 kilobase pair tandemly repeated DNA, as shown by Southern blot analysis and DNA cloning. Thus, the rRNA operon of this eukaryotic organism can be no longer than a typical bacterial operon. Sequence analysis identified the 127 nucleotide RNA as homologous to 5.8S RNA, but comparisons to archaebacterial rRNA suggest that Giardia derived from an early branch in eukaryotic evolution.

INTRODUCTION

Ribosomal RNA (rRNA) structural analysis has emerged as an important tool for phylogenic analysis (1). Recent studies using this approach have demonstrated that lower eukaryotes, especially the protozoa, are surprisingly diverse (2). Nevertheless, one aspect of rRNA structure has remained relatively consistent in evolution: size. Bacteria possess 5S, 16S, and 23S rRNA, with typical lengths of 118, 1500, and 2900 nucleotides, respectively. The analogous components of the cytoplasmic ribosomes of eukaryotes are typically 119, 1800, and 3400-4700 nucleotides long; in addition they usually include a 5.8S RNA about 160

nucleotides long (3-5). Until recently, significant deviation from these typical lengths had been seen only in the rRNA of mitochondria. Unusually large (6) and small (7) 16S-type RNA have now been characterized in two different protozoa; in addition, 5.8S RNA is reportedly absent in one of these (8).

We have been characterizing on a molecular level Giardia lamblia, the most frequently identified intestinal parasite in the U.S. and an important cause of diarrheal disease worldwide (9,10). G. lamblia is an anaerobic protozoan, devoid of mitochondria or kinetoplasts but possessing two nuclei, flagella, and a unique structure termed the median or parabasal body. In the course of isolating messenger RNA from G. lamblia, a large amount of rRNA was apparent. Upon further examination, this RNA was revealed to be unusually small for the major rRNA of a eukaryotic or even prokaryotic cell. Hybridization analysis and cloning of a rRNA gene revealed it to be correspondingly small as well. Sequence analysis identified an abundant 127 nucleotide RNA as homologous to 5.8S RNA, with about 60% similarity to both other protozoal and to archaebacterial rRNAs. Preliminary results from this study have been previously reported (11).

MATERIALS AND METHODS

Cell culture

G. lamblia strains WB, PO, RS, and LT (12) were obtained from Dr. Philip Smith, NIH. Trophozoites were cultured at 37°C in Diamond's TYI-S-33 supplemented with 10% fetal bovine serum, bovine bile (0.5 mg/ml) and L-cysteine (2 mg/ml) (13).

RNA and DNA preparation

Non-attached cells from late-log cultures were discarded, and the attached cells were washed in cold phosphate-buffered saline. Total nucleic acids were isolated by phenol extraction in the presence of 0.5% sodium dodecyl sulfate (SDS). Following ethanol precipitation, the nucleic acids were suspended in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA (TE) and stored at 4°C. Non-degraded RNA was also isolated by solubilization of cells in guanidinium isothiocyanate followed by cesium chloride ultracentrifugation (14). After phenol extraction, the purified RNA was stored as an ethanol precipitate at -20°C. Non-

polyadenylated RNA was isolated by chromatography through oligo(dT) cellulose, and stored as an ethanol precipitate. Ribosomal pellets were prepared by homogenizing trophozoites in cold ribosome buffer (10 mM magnesium acetate, 100 mM NH4Cl, 20 mM Tris-HCl, 2 mM 2-mercaptoethanol, pH 7.5) containing 0.5% sarkosyl, or by 10 cycles of freezing and thawing in the same buffer (without sarkosyl), until lysis was apparent microscopically. Debris and nuclei were removed by centrifugation at 3,000 x g for 10 min, and the supernatants were further centrifuged at 100,000 x g for 3 h. The pellet was suspended in ribosome buffer, diluted with TE + SDS, and extracted with phenol. The rRNA was stored as an ethanol precipitate.

Gel electrophoresis

Non-denaturing and formaldehyde-formamide agarose gels (1% except Fig. 5) were prepared as described (14). In all cases, 5-10 µg of nucleic acid were analyzed. Non-denaturing gels were stained with ethidium bromide and formaldehyde-formamide gels were stained with acridine orange. For size analysis of low molecular weight RNA, an 8% polyacrylamide, 7 M urea sequencing gel (15) was used. Following electrophoresis, the gel was exposed to X-ray film to visualize end-labelled RNAs. For DNA restriction, the conditions specified by the enzyme's manufacturer were used, and for hybridization analysis the method of Southern (14) was employed.

RNA labelling

RNA was purified by elution from agarose or polyacrylamide gel slices in TE + SDS overnight at 45°C, followed by ethanol precipitation. The purified RNA was dephosphorylated with calf intestinal phosphatase (Boehringer Mannheim) (14), followed by phenol extraction and ethanol precipitation. RNA was 5'-end labelled with ^{32}P-ATP using T4 polynucleotide kinase (Pharamacia), or 3'-end labelled with ^{32}P-pCp using T4 RNA ligase (Bethesda Research Labs), to specific activities of 10^7-10^8 cpm per µg.

DNA cloning

Total G. lamblia DNA was cleaved with BglII, and ligated to BamHI-cleaved pUC19 vector DNA. After transformation of

Escherichia coli strain TB1, 142 clones with inserts were screened with end-labelled rRNA by the procedure of Grunstein and Hogness (14). Plasmid DNA from two of the 10 hybridizing clones was prepared (16) and analyzed by restriction digestion, Southern blotting, and probing with individual gel-purified end-labelled rRNAs. The two clones (B4 and C4) were inserted in opposite orientation but had identical restriction patterns. Subclones for DNA sequencing were obtained by digestion of B4 DNA with CpfI (Cooper Biomedical) and ligation into BamHI-cleaved M13mp18 RF DNA, followed by transfection of E. coli JM109. Plaques were screened for hybridization to end-labelled rRNA. The 5'-3' orientation of rRNA encoded within B4 and C4 was deduced by hybridization of a rRNA probe to one of the M13 single-stranded DNA clones.

RNA and DNA sequencing

Partial sequences of G. lamblia 127 nucleotide RNA were determined by the enzymatic method (17), using both 5' and 3' end-labelled, gel-purified RNA. The DNA sequence of a CpfI fragment cloned in M13mp18, and identified by hybridization with end-labelled 127 nucleotide RNA, was determined by the dideoxy method (18).

RESULTS AND DISCUSSION

Non-denaturing agarose gel electrophoresis of total nucleic acids extracted from axenically cultured trophozoites of G. lamblia strain WB revealed the expected high molecular weight DNA (Fig. 1A), along with a large amount of rRNA. We estimate there are 8 x 10^5 copies of each rRNA molecule per cell, based on a yield of 50 A_{260} units from 2.8 x 10^7 cells, with rRNA representing about 95% of this material. The products of digestion with the restriction enzyme BglII are also shown in Fig. 1A. This digestion generated one prominent DNA band, visible above the background of random fragments. Approximately 1% of the total DNA is represented in this fragment; this corresponds to 300 copies per cell, based on a DNA content of 1.6 x 10^8 bp per cell (our unpublished data and R.A. Alonso, personal communication). Subsequent analysis (see below) identified this fragment as the rRNA gene, which in other protozoa is similarly

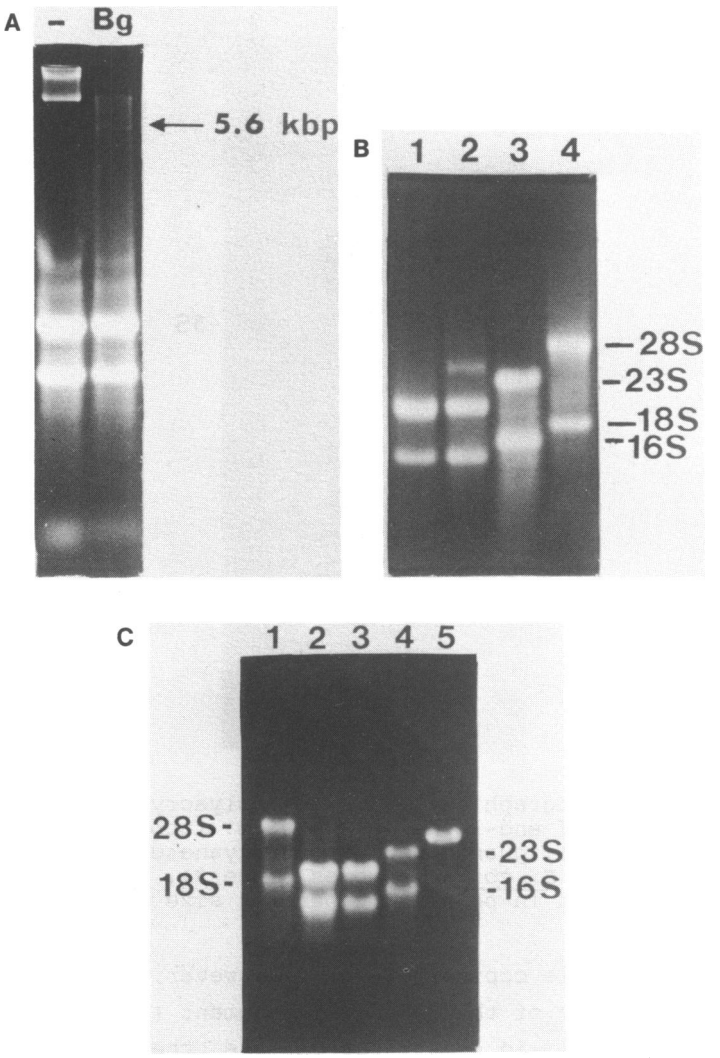

Fig. 1. (A) Non-denaturing agarose gel electrophoreis of total G. lamblia nucleic acid, untreated (-) and cleaved with BglII (Bg). The repetitive DNA fragment at 5.6 kbp is indicated; HindIII-lambda DNA was run for size markers. (B) Non-denaturing agarose gel electrophoresis of: phenol extracted RNA from G. lamblia ribosome pellet (lane 1); guanidinium isothiocyanate extracted G. lamblia total RNA (lane 2); E. coli rRNA (lane 3); rabbit reticulocyte rRNA (lane 4). (C) Formaldehyde-formamide agarose gel electrophoresis of: rabbit reticulocyte rRNA (lane 1); G. lamblia total RNA (lane 2); G. lamblia poly(A-) RNA (lane 3); E. coli rRNA (lane 4); MS2 bacteriophage RNA (lane 5). Sizes of standards (in nucleotides): MS2 (3570), E. coli 16S (1540) and 23S (2900), rabbit reticulocyte 18S (1860) and 28S (4700).

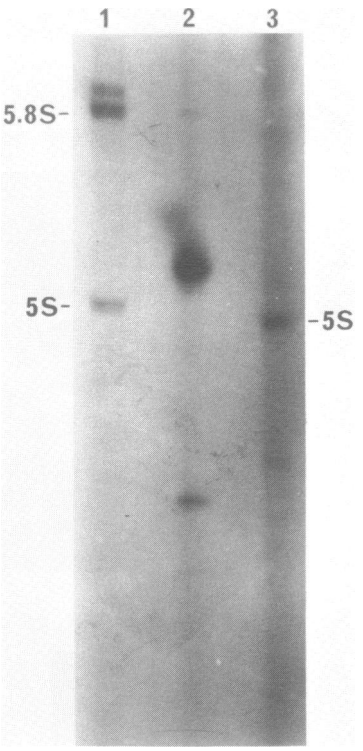

Fig. 2. Autoradiograph of 7M urea/8% polyacrylamide gel electrophoresis of end-labelled RNA from: rabbit reticulocyte ribosomes (lane 1); guanidinium isothiocyanate-extracted G. lamblia (lane 2); E. coli ribosomes (lane 3). DNA sequencing reactions were run in parallel lanes for size markers.

present in multiple copies per cell. However, unlike other protozoa, the size of the rRNA gene fragment (5.6 kbp) appears to be unusually small; in yeast, for example, the rRNA repeat unit is 10 kbp long (for review, see 19). We therefore undertook a further investigation of this gene fragment and of the rRNA itself.

Size analysis of G. lamblia RNA

A 100,000 x g ribosome pellet was prepared from cells lysed in ribosome-stabilizing buffer. Phenol extraction of the pelleted ribosomal material followed by non-denaturing agarose gel electrophoresis revealed two major high molecular weight RNA species (Fig. 1B, lane 1). These correspond to the 16/18S and

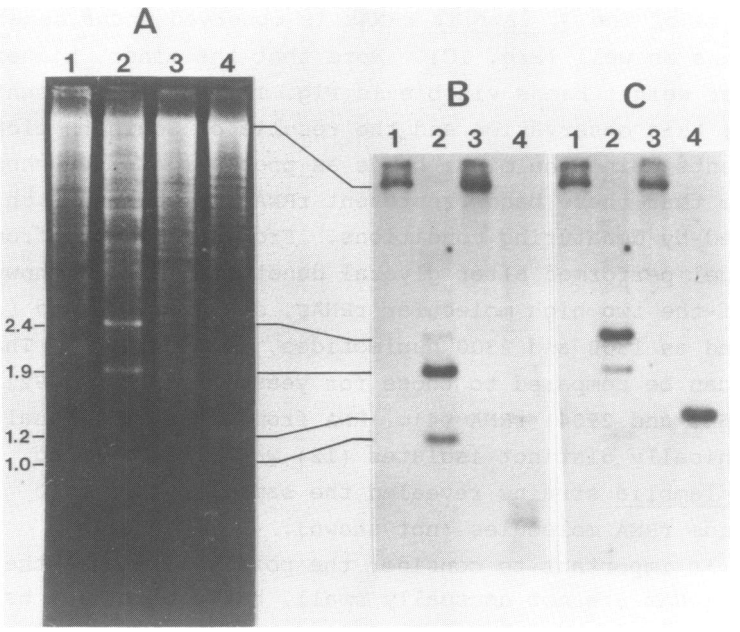

Fig. 3. Agarose gel electrophoresis of G. lamblia total DNA stained with ethidium bromide (A) and Southern blotted followed by probing with end-labelled L (B) or S (C) RNA and autoradiography. DNA was cleaved with EcoRI (lane 1), BamHI (lane 2), SalI (lane 3), or SmaI (lane 4). Indicated fragment lengths (in kbp) were determined by comparison to HindIII-lambda DNA markers.

23/28S RNA components of bacterial and eukaryotic ribosomes. The relative intensity of the two species is consistent with their presence in equal numbers, as expected for rRNA. Although both species migrated considerably faster than rRNA standards from E. coli and rabbit reticulocytes, size estimates cannot be accurately made from non-denaturing gels. Furthermore, the conditions used to isolate ribosomes do not guard against ribonuclease nicking of the rRNA, which could account for the small size of these molecules.

Trophozoites were therefore extracted directly with the potent nuclease inhibitor guanidinium isothiocyanate, and RNA was analyzed by both non-denaturing and formaldehyde/formamide gel electrophoresis. Both RNA preparations generated the same two major bands (Fig. 1B, lanes 1 and 2); furthermore, the relatively

small size of the G. lamblia rRNAs is observed under denaturing conditions as well (Fig. 1C). Note that the minor, higher molecular weight bands visible in Fig. 1A and B are absent in Fig. 1C; this observation and the results of Southern blotting experiments using the minor bands as probes (data not shown) indicate that these bands represent rRNA aggregates which are disrupted by denaturing conditions. From Fig. 1C and from a second gel performed after glyoxal denaturation (not shown), the sizes of the two high molecular rRNAs, designated S and L, were estimated as 1300 and 2300 nucleotides, respectively. These values can be compared to those for yeast (1799 and 3392) and E. coli (1542 and 2904) rRNA (4). RNA from three additional geographically distinct isolates (12) was also examined. All four G. lamblia strains revealed the same 1300 and 2300 nucleotide rRNA molecules (not shown).

It is important to consider the possibility that the G. lamblia rRNAs are not unusually small, but rather have been endonucleolytically processed, as is the 26S rRNA species of many invertebrates (5). The gel electrophoretic patterns shown in Fig. 1 clearly rule this out, since no additional bands down to the resolution limit of 200 to 300 nucleotides can be observed (see also Fig. 2 below). Furthermore, the observed bands cannot be unresolved dimers of two different fragments; when used as probes in Southern blotting experiments, only the appropriately sized DNA fragments hybridized (see Figs. 3 and 5 below).

It was also of interest to examine the low molecular weight RNA components of G. lamblia. Non-polyadenylated RNA isolated from cells solubilized with guanidinium isothiocyanate was 5' end-labelled with ^{32}P. Electrophoresis through a polyacrylamide sequencing gel containing 7M urea and autoradiography revealed one major low molecular weight RNA band, 127 nucleotides long (±1 nucleotide, relative to DNA sequencing markers) (Fig. 2). A 93 nucleotide RNA and additional minor species from 30 to 80 nucleotides long were also observed; there were no bands of significant intensity above 130 nucleotides to the 400 nucleotide resolution of the gel. An identical pattern was seen in autoradiographs of 3' end-labelled RNA, and in ethidium bromide stained gels (data not shown). On some gels, the 127 nucleotide

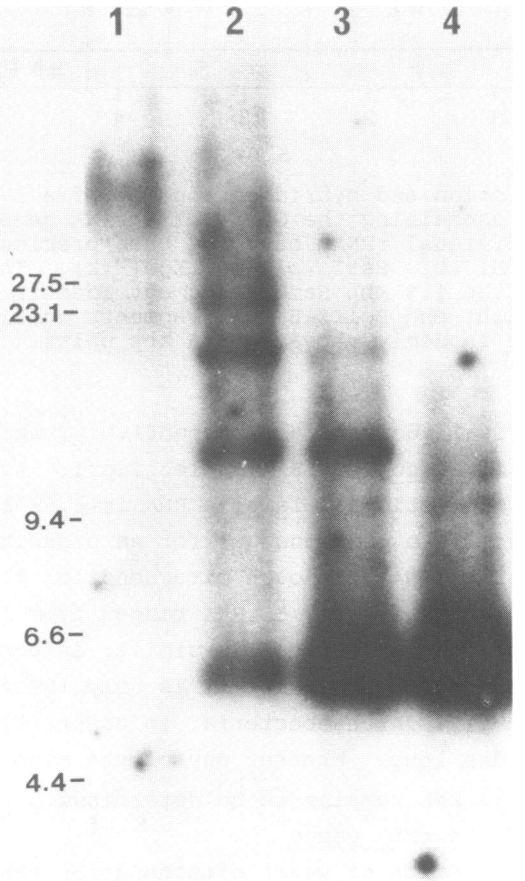

Fig. 4. Autoradiograph of Southern blot from 0.3% agarose gel electrophoresis of BglII-digested G. lamblia chromosomal DNA, probed with end-labelled G. lamblia L RNA. Digestion was with 2 units enyzme per µg DNA for: 0 min(lane 1); 1 min (lane 2); 3 min (lane 3); 9 min (lane 4). HindIII-lambda DNA was run for size markers.

RNA was resolved into a doublet. However, in phenol-extracted ribosomal pellets, a 113 nucleotide species was also present. This band may be a degradation product of the 127 nucleotide RNA, since in different preparations the intensity of the two bands is inversely related. For comparison, rabbit reticulocyte and E. coli rRNAs were also analyzed. Typical eukaryotic 5.8S and 5S

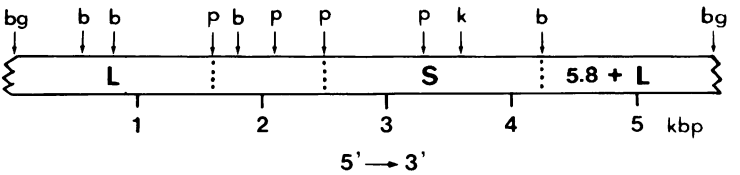

Fig. 5. Restriction and hybridization map of a 5.6 kbp cloned BglII fragment containing the G. lamblia rRNA genes. 5' and 3' ends of the individual rRNAs have not been precisely determined. BglII (bg), BamHI (b). PstI (p), and KpnI (k). The BglII site is located within the 1.9 kbp BamHI fragment identified in Fig. 3; the left and right end BglII-BamHI fragments combine to form this fragment on the tandemly repeated 5.6 kbp units.

RNAs (158 and 119±1 nucleotides, respectively) were apparent, as was the 5S RNA of E. coli (118±1 nucleotides). As described below, the 127 nucleotide G. lamblia RNA is 5.8S-like in sequence. It would be most unusual for an organism to lack the highly conserved 5S RNA, although mitochondrial ribosomes appear to do so (20). In eukaryotes 5S RNA ranges from 116-122 nucleotides long; the size range is similar in eubacteria, with the exception of Mycoplasma where it is only 107 nucleotides (for compilation see 21). Archaebacteria, in contrast, have 5S RNA up to 131 nucleotides long. Whether any of the minor RNA species represent 5S-like RNA remains to be determined.

Organization of the rRNA genes

With the exception of yeast mitochondrial rRNA, and the 5S RNA of many eukaryotes, the multiple rRNA species derive from a single precursor RNA by endonucleolytic processing (19). This is almost certainly the case with G. lamblia as well, since the single 5.6 kbp genomic DNA fragment generated by BglII (Fig. 1A) hybridized in Southern blot analysis to individually purified S, L, and 127 nucleotide rRNA probes (data not shown). Analysis with four additional restriction enzymes is shown in Fig. 3. The three BamHI fragments (lane 2), plus a fourth fragment (0.2-0.3 kbp) not visible on this gel, total 5.6 kbp, identical to the size of the BglII fragment. A large number of SmaI cleavage sites (recognition sequence: CCCGGG) are present (lane 4; average fragment size about 0.5 kbp), suggesting an unusually high G+C content for the rRNA genes. In contrast, the EcoRI (lane 1) and

```
C.r.        5'-AAC--UCUCAAC--AACGGAU--AUCUUGGCUC-U-CGGAUCGAUGAAGGACGCAGCGAAAUGCGAUA-
                ***  ** *  *    *****   **  *** *   ***  *** **** *** ****    *** *
G.l. 5'-GCCCCCGAAC-GUCCCCGCC-GGCGGAU-GCUC--GGCCCGGGCGG--CGACGAAGAGCGCGGCGA----CGAGA-
               ** ** **  ** ** **** ****  *** ***   *  ********  **  ** *     *
H.c.  5'-GUGGCUACUGUGCC-ACCUGGUGGAUAGCUC--GGCUCGGAU-GC-CGACGAAGGACG-UGCCA----AGCUG-

C.r. -CGUAGUG--UGA--ACUGCAGAAAUACGUGAACUAUCGAAUCCCUGAACGU-AUACUGC-GC--CCGAGGCCCC-(3')
      **  ***   **  ** **       ** ***  * ***  ****   **  ******* **  **  *****
G.l. -CGCGGUGC-GGAGCAC-GCCACGCC-CGAGAAGCA-CGA--CCCU--CCG--AUACUGCAGCUGCC-CGGCCCU-3'
      **    **  ***    *** * *   ***    *  **    **  * *  ** ** *   **      ****
H.c. -CGAUAAGCCUGAGGGA-GCCGCAC--GGAGGCUAA--GA--A-CU--CAG-GAU-CUCCUAAUGGG-AAUCCCU-(3')
```

Fig. 6. Sequence of the 127 nucleotide RNA from G. lamblia (G.l.). The 5' and 3' ends have not been precisely defined. Asterisks denote conserved nucleotides between G. lamblia and the 5.8S rRNA from Chlamydomonas reinhardi (C.r.) or the 5' terminus of 23S rRNA from Halobacterium cutirubrum (H.c.).

SalI (lane 3) fragments hybridizing with rRNA probes comigrated with uncleaved genomic DNA. The explanation for this was revealed by the Southern blot analysis shown in Fig. 4. Digestion of genomic DNA with a limiting amount of BglII for varying lengths of time followed by probing with labelled rRNA produced a ladder of bands with approximate 5.6 kbp spacing. Thus, the rRNA operons of G. lamblia are apparently organized in head to tail tandem repeats of 5.6 kbp units.

The structure of the G. lamblia rRNA operon was further defined by DNA cloning of the 5.6 kbp BglII fragment from a genomic library constructed in pUC19. A restriction map is shown in Fig. 5, with the approximate locations and 5'-3' orientation of the L, S, and 127 nucleotide rRNA species as determined by Southern blot analysis. A substantial (0.9 kbp) region which failed to hybridize to any of the mature rRNA sequences is located between the L RNA 3' end and the S RNA 5' end. This is the expected location for the intergenic region extending from the transcriptional termination site to the promoter. The 127 nucleotide RNA maps to the fragment encoding the 5' end of L RNA; this is the usual location of 5.8S RNA. As with the rRNA molecules themselves, the G. lamblia rRNA operon, which must be less than 5.6 kbp long, is surprisingly small. For comparison, the rRNA precursors encoded within the operons of yeast and E. coli are 7200 and 5600 nucleotides long, respectively.

Sequence of a 5.8S-like RNA

The most abundant low molecular weight RNA molecule, the 127 nucleotide species, was terminally labelled and subjected to RNA sequencing by the enzymatic method (17). The sequence obtained was further compared to the DNA sequence of an M13 subclone, derived from the cloned BglII fragment and isolated using the 127 nucleotide RNA as a probe. As predicted from the results of SmaI digestion (Fig. 3), the G (36%) and C (41%) base contents are unusually high. Comparisons of the derived sequence to a large number of rRNA sequences from other organisms (see 5 for references) revealed the 127 nucleotide RNA to be homologous to the 5.8S component. As shown in Fig. 6, the degree of similarity to 5.8S RNA of Chlamydomonas reinhardi is 66%; other protozoa are somewhat less (typically 60%). The alignment shown suggests that the 127 nucleotide RNA is colinear with 5.8S RNA; thus, the reduced size of the G. lamblia RNA relative to other 5.8S RNAs results from processing of the RNA precursor at an alternative site, and not from a deletion within the 5.8S RNA gene. Interestingly, processing at a similar location is used to generate the 123 nucleotide 5.8S RNA of Drosophila melanogaster (21).

Since 5.8S RNA evolved from the 5' terminus of bacterial 23S RNA (22), we also compared the 127 nucleotide RNA to several 23S RNA sequences. There is a significant level of similarity (60%) to archaebacterial 23S RNA (23,24) (Fig. 6) but much less to eubacterial RNA; similar results are obtained for comparisons made with Chlamydomonas 5.8S RNA. It has been previously noted that in a number of ways eukaryotes and archaebacteria appear to be more closely related than eukaryotes and eubacteria (25). The comparisons of the 5.8S-like RNAs, and additional comparisons made with partial sequences of the S and L RNAs (unpublished data) consistently indicate that Giardia sequences have diverged about equally from other protozoa as from archaebacteria. This suggests that the Giardia line of descent branched early in eukaryotic evolution. Recently, an evolutionarily primitive position for those protozoa lacking mitochondria, including Giardia, has been proposed (7,26). Further studies to delineate the evolutionary and functional significance of the unusually small Giardia rRNA molecules are warranted.

Note: After this manuscript was submitted, Boothroyd et al. (27) reported results very similar to ours for the Portland 1 strain of G. lamblia.

ACKNOWLEDGEMENTS

We thank P. D. Smith and D. E. Keister for providing G. lamblia and advice on cultivation, and M. Agarwal for excellent technical assistance.

REFERENCES

1. Pace, N.R., Olsen, G.J., and Woese, C.R. (1986) Cell 45,325-326.
2. Sogin, M.L., Elwood, H.J., and Gunderson, J.H. (1986) Proc. Natl. Acad. Sci. USA 83,1383-1387.
3. Delihas, N., Andersen, J., and Singhal, R.P. (1984) Prog. Nucl. Acid Res. Mol. Biol. 31,161-190.
4. Noller, H.F. (1984) Ann. Rev. Biochem. 53,119-162.
5. Brimacombe, R. and Stiege, W. (1985) Biochem. J. 229,1-17.
6. Gunderson, J.H. and Sogin, M.L. (1986) Gene 44, 63-70.
7. Vossbrinck, C.R., Maddox, J.V., Friedman, S., Debrunner-Vossbrinck, B.A., and Woese, C.R. (1987) Nature 326,411-414.
8. Vossbrinck, C.R. and Woese, C.R. (1986) Nature 320,287-288.
9. Meyer, E.A. and Jarroll, E.L. (1986) Am. J. Epidemiol. 111,1-12.
10. Stevens, D.P. (1985) Rev. Inf. Dis. 7,530-535.
11. Edlind, T.D. and Chakraborty, P.R. (1986) American Society of Parasitologists Annual Meeting, Abtract 141.
12. Smith, P.D., Gillin, F.D., Kaushal, N.A., and Nash, T.E. (1982) Infect. Immun. 36,714-719.
13. Keister, D.E. (1983) Trans. R. Soc. Trop. Med. Hyg. 77,487-488.
14. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.
15. Sanger, F. and Coulson, A.R. (1978) FEBS Lett. 87,107-110.
16. Holmes, D.S. and Quigley, M. (1981) Anal. Biochem. 114,193-197.
17. Donis-Keller, H. (1980) Nuc. Acids Res. 8,3133-3142.
18. Sanger, F., Nicklen, S., and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74,5463-5468.
19. Mandal, R.K. (1984) Prog. Nuc. Acid Res. Mol. Biol. 31,115-160.
20. Thurlow, D.L., Mason, T.L., and Zimmermann, R.A. (1984) FEBS Lett. 173,277-282.
21. Pavlakis, G.N., Jorden, B.R., Wurst, R.M., and Vournakis, J.N. (1979) Nuc. Acids Res. 7,2213-2238.
22. Nazar, R.N. (1980) FEBS Lett. 119,212-214.
23. Hui, I. and Dennis, P.P. (1985) J. Biol. Chem. 260,899-906.
24. Jarsch, M. and Bock, A. (1985) Mol. Gen. Genet. 200,305-312.
25. Zillig, W., Schnabel, R., and Stetter, K.O. (1985) Curr. Top. Microbiol. Immunol. 114,1-18.
26. Cavalier-Smith, T. (1987) Nature 326,332-333.
27. Boothroyd, J.C., Wang, A., Campbell, D.A., and Wang, C.C. (1987) Nucl. Acids Res. 15,4065-4084.