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A single-stranded RNA copy of the *Giardia lamblia* virus double-stranded RNA genome is present in the infected *Giardia lamblia*

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ABSTRACT

An isolate of *Giardia lamblia* infected with the double-stranded RNA virus (GLV) has two major species of RNA that are not present in an uninfected isolate. One of these species is the previously characterized double-stranded RNA genome of GLV (1). The second species of RNA appears to be a full length copy of one strand of the double-stranded RNA genome. This full length single-stranded RNA is not present in viral particles isolated from the growth medium. The cellular concentration of the single-stranded RNA changes during exponential and stationary phases of cell growth in a fashion consistent with a viral replicative intermediate or mRNA. The single-stranded species does not appear to be polyadenylated.

INTRODUCTION

Giardia lamblia is a flagellated protozoan parasite. The *G. lamblia* trophozoite lives in the small intestines of mammalian hosts and is responsible for many cases of debilitating and chronic diarrhea worldwide. As many as 7% of the United States population are asymptomatic carriers. *G. lamblia* contaminates water supplies in its infective cyst form. The parasite infection is most commonly treated with the drug metronidazole though the drug has common side-effects and potential carcinogenicity. Therefore, the development of new chemotherapeutic agents is a necessity (reviewed in 2).

The *G. lamblia* virus (GLV) is a double-stranded (ds) RNA virus which infects many isolates of *G. lamblia* (3). The virus is composed of a single major 100 kd coat protein which encapsulates a 7 kb dsRNA (4). The virus appears to replicate without inhibiting growth or harming the *G. lamblia* trophozoites. It is also extruded into the culture medium. The extruded virus can infect many other isolates of the protozoan which do not contain the virus, though there are isolates of the parasite which are resistant to infection by GLV (3). The virus does not seem to be associated with the virulence of the parasite (4). GLV has not been

observed in the cyst form of the parasite and we have not yet determined whether the virus can be carried through the transformation between cyst and trophozoite. The structural features of GLV and the stable replication of GLV inside the parasite are similar to the *Saccharomyces cerevesiae* dsRNA killer virus and the many related fungal viruses (5, 6). Unlike the yeast virus, isolated GLV can infect uninfected *G. lamblia*. The yeast virus can only be transmitted by cell division and mating.

GLV is the first virus of a parasitic protozoan origin clearly identified and purified in its infectious form. Presently, there are no genetic vectors for introducing nucleic acids into the parasitic protozoa. Therefore, GLV is a potential genetic vector in *G. lamblia*. However, the viral genome is a dsRNA which makes genetic manipulations difficult. Therefore, cloning of a full length cDNA and reconstitution of the viral particle may be necessary to construct a useful transfection vector. It is technically difficult to synthesize a cDNA from a dsRNA. In our efforts to obtain this clone we have identified a full length single-stranded (ss) RNA copy of the viral genome in total RNA extracts of the parasite. The isolation of this ssRNA will undoubtedly improve our chances of obtaining a full length cDNA of GLV.

MATERIALS AND METHODS

Materials

RNAse U2 was obtained from Pharmacia. HindIII cut λDNA, pBR322, MspI, yeast tRNA, salmon sperm DNA, T7 and T3 RNA polymerases were obtained from Bethesda Research Laboratories. Nitrocellulose filters were obtained from Schleicher and Schuell. All radionucleotides were obtained from Amersham with the highest possible specific activities. Ficoll and glycerol loading dyes were prepared as described (7).

Cells

Portland I and WB strains of *G. lamblia* were cultivated *in vitro* as described previously (3). Portland I harbors GLV, whereas WB is an uninfected isolate which is susceptible to infection with GLV.

Nucleic Acids

Nucleic acid extracts of *G. lamblia* strains were prepared as previously described (8). The GLV dsRNA genome was prepared from virus particles (1). Nucleic acid species which were purified by agarose or polyacrylamide gel electrophoresis were eluted from the respective gel slice by the Schleicher and Schuell Elutrap device according to the instructions provided.

GLV dsRNA Depleted Portland I Total RNA

A solution of Portland I total RNA (400 µg/ml) was diluted 1:10 with a solution of 18 mM Tris-borate (pH 8.3), 1 mM ethylenediamine tetraacetic acid (EDTA), 150 mM NaCl, 35% ethanol. The solution was allowed to stand at room temperature for 30 minutes and the resultant precipitate was removed by centrifugation. The supernatant fluid was collected and diluted to a final ethanol concentration of 71% and cooled to -70°C for 10 minutes. The precipitate was collected by centrifugation. Both the 35% and the 71% ethanol precipitates were washed with 95% ethanol and dried in a vacuum centrifuge. Finally, the precipitates were dissolved in diethylpyrocarbonate (DEPC) treated water. The two samples were analyzed by agarose gel electrophoresis and ethidium bromide staining. The low ethanol precipitate contains primarily the ribosomal RNA's and SS (the presently characterized viral RNA species). It is largely depleted of tRNA and completely depleted of GLV dsRNA and DNA. The high ethanol precipitate contains most of the tRNA, the DNA, and GLV dsRNA.

3' End Probe Preparation

GLV dsRNA was labeled at the 3' end with (^{32}P)pCp (9). The labeled RNA had a specific activity of 10^6 cpm/mg. The labeled RNA (2 µg) was treated with 5 units of RNAse U2 in 100 µl of DEPC treated sodium citrate (25 mM) for 30 minutes at 55°C. The reaction was quenched by the addition of 30 µl of dimethylsulfoxide (DMSO) and heated to 90°C for three minutes then cooling on ice for 1 minute. The sample was mixed with glycerol loading dye and electrophoresed for 90 minutes on a 7% polyacrylamide urea gel (0.2mm x 400mm x 150mm) at a constant 1700 V. Autoradiography of the gel was performed for 30 minutes at room temperature. The resultant autoradiogram showed two major bands of radioactivity migrating between 150 and 170 nucleotides, based on a labeled size marker of pBR322 cut with MspI, treated with calf intestinal phosphatase then kinased with T4 polynucleotide kinase and γ-(^{32}P)ATP. The two major bands were excised separately from the gel and eluted from the gel slice with 10 µg of yeast tRNA as carrier using the elutrap method as described by the manufacturer. Yields of radioactivity recovered in these fragments range from 50,000 to 200,000 cpm.

Partially Hydrolyzed and Kinased GLV dsRNA Probe

GLV dsRNA (100 ng) was treated with 100mM Na_2CO_3 in a total volume of 20 µl for 5 minutes at 90°C (10). The partially hydrolyzed RNA was ethanol precipitated then kinased with γ-(^{32}P)ATP (10 mCi/mmole) and T4 polynucleotide kinase according to the directions of the manufacturer. The yield of labeled RNA was 10^7 cpm.

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   1
   AAGCGGATAACGAGTGGATACCGACTTCAGGGCCTGCTTGGAAGGTACCATATCTGGAAA
61
   ACGTAGTTAAGCGGTCTGGCAGGCGCTTGCTGGCGGAGCTCAGGATAGCATCCAATAACG
121
   GGTCTGGAGACCGTACCTTTCTTGACGACGTGATAGACAAGAAAGGGAATGCATTTTGCT
181                                                    211
   ACTTCTCTGCTGCTTTGGGGGGCAAGATCCT
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Figure 1. Sequence of pG30: Clone of the Viral dsRNA.

The sequence was obtained as described in the Methods. The underlined region is the sequence of a DNA oligonucleotide chemically synthesized. The reverse complement oligonucleotide was also synthesized.

Electrophoresis

Agarose and polyacrylamide gel electrophoresis were performed as described (7).

Transfers of Nucleic Acids to Nitrocellulose Filters

Nucleic acids were transferred from agarose gels to nitrocellulose by a modification of the procedure developed for the yeast virus dsRNA (Jeremy Bruenn, personal communication). The gel was first treated with 50% formamide and 17% formaldehyde at 55°C for 1 hour, washed with H_2O, soaked in 50 mM NaOH and 10 mM NaCl for 45 minutes, neutralized by soaking in 0.1 M Tris-HCl (pH 7.5), and finally soaking in 20X SSC for 1 hour. The RNA was then transfered to the nitrocellulose in 20X SSC. Labeled (^{32}P) T7 and T3 RNA polymerase transcripts from pG30 (detailed below) were prepared as described in the Stratagene Bluescript vector instruction manual. Hybridizations with RNA probes were performed by prehybridizing the filter in 50% formamide, 5X sodium chloride-sodium phosphate-EDTA (SSPE), 5X Denhardts, 0.2% sodium dodecyl sulfate (SDS), 0.1 mg/ml salmon sperm DNA (sonicated (7)), and 0.1 mg/ml yeast tRNA at 55°C for 3 hours. After prehybridization, the filter was hybridized to the labeled RNA for 18 hours under the same conditions. Finally, the filters were washed with 3X saline sodium citrate (SSC), 0.1% SDS 6 times for 20 minutes at 65°C, then exposed to film. Hybridizations with DNA oligonucleotides labeled by kinasing with γ (^{32}P)ATP and T4 polynucleotide kinase were performed as described (10).

Molecular Cloning of a Partial GLV cDNA

Approximately 1 µg of GLV dsRNA was obtained from CsCl -gradient purified virus particles (4). The dsRNA was denatured in 40 mM methyl mercury (Thiokol) for 10 minutes at room temperature, then diluted into 10 volumes of reverse transcriptase reaction mixture as provided by the Amersham

cDNA synthesis kit. First and second strands of cDNA were then synthesized according to the supplier's instructions except that random hexamer nucleotides (Pharmacia) were used as primers for the first strand synthesis. The cDNA was ligated with EcoR1 linkers (Pharmacia), digested with EcoR1 and then ligated into the Bluescript vector (Stratagene).

A modified dideoxy sequencing method adapted for the use with supercoiled plasmids and (^{35}S)dATP was employed to determine the nucleotide sequence of our partial cDNA clone (12). Reverse transcriptase (13) and 7 - deazaguanosine (14) were also substituted for Klenow fragment and dGTP in some of the reactions in order to produce a more readily interpreted sequence ladder.

Viral Infection Time Course

At time 0, cells from strain WB (which does not contain GLV) were infected with CsCl purified GLV (4) at a multiplicity of infection (M.O.I.) of 1,000. The infected cells were used to inoculate several tubes at an initial cell concentration of 10^5 cells/ml. At the time points indicated, an appropriate number of tubes were chilled on ice for 20 min and inverted several times to dislodge the attached *Giardia*. A cell count was then taken on the sample using a Coulter Counter as described (4) and the cells were removed by centrifugation. The medium from the sample was filtered through a 0.22μ filter from Schleicher and Schuell to remove any remaining cells. The virus was isolated from the medium by centrifugation at 346,000 x g for 45 min in a Ti-100.3 rotor (Beckman). The viral pellet was resuspended in phosphate buffered saline (PBS) with 0.5% SDS and incubated with proteinase K (100 μg/ml) for 1 hr. at 37°C. The viral RNA was then purified by extractions with phenol, phenol/chloroform, and chloroform and then ethanol precipitated. The cells from each time point were washed and resuspended in PBS, lysed with 0.5% SDS and hot phenol, and purified in the same way that the viral RNA was purified (except that the proteinase K step was omitted).

Samples of the viral and total RNAs were electrophoresed through a 0.8% agarose gel in 1X TBE with 0.5 μg/ml of ethidium bromide, using λDNA cut with Hind III as a standard marker.

RESULTS

Partial cDNA Clone of GLV dsRNA (pG30)

The recombinant plasmid obtained from our cloning procedure contained an insert of 210 bp. Its nucleotide sequence is given in Figure 1. The recombinant plasmid was subsequently verified by hybridization experiments using

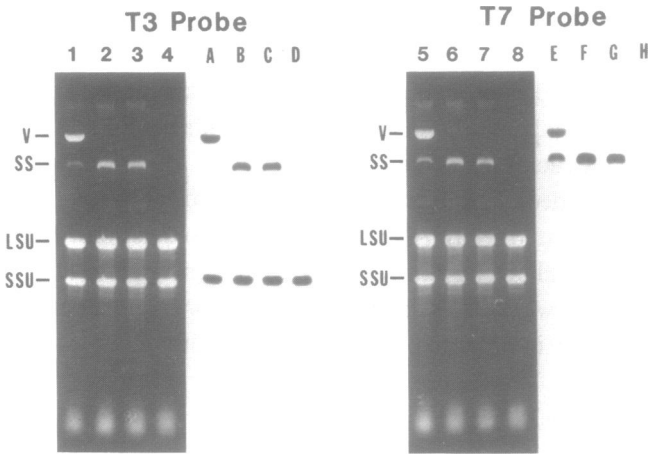

Figure 2. Northern Analysis of Native and Denatured *G. lamblia* Total RNAs.

Lanes 1 - 8 are the ethidium bromide stain of RNA (10 mg/lane) electrophoresed in a 0.8% agarose gel. Lanes 1 and 5 are *G. lamblia* strain Portland I total RNA, lanes 2 and 6 are *G. lamblia* strain Portland I total RNA denatured with 10% formamide (90°C, 5 minutes), lanes 3 and 7 are *G. lamblia* strain Portland I total RNA denatured with 40% formamide (90°C, 5 minutes), lanes 4 and 8 are *G. lamblia* strain WB total RNA. The RNA in lanes 1 - 8 were transferred to nitrocellulose, and the filters were hybridized at 55°C to RNA transcripts (107 cpm, 0.2 mCi/nmole CTP) from pG30, followed by autoradiography. Lanes A - D correspond to lanes 1 - 4 after hybridization with the T7 transcript, lanes E - H correspond to lanes 5 - 8 after hybridization with the T3 transcript. Viral dsRNA (V), viral ssRNA (SS), small subunit ribosomal RNA (SSU), large subunit ribosomal RNA (LSU).

hydrolyzed, kinased, (^{32}P)-labeled GLV dsRNA as a probe. The nucleotide sequence has only one open reading frame, and computer-genertated amino acid sequence does not bear any significant homology to known peptide sequences in the Dayhoff Data Base. We have not yet determined what portion of the GLV genome the clone was derived from.

Characterization of the Viral ssRNA

Comparison of the total RNA from a strain of *G. lamblia* containing GLV (Portland I) with the total RNA of a strain which does not contain the virus (WB), by agarose gel electrophoresis, demonstrated that two major species of RNA were present in the infected strain but not in the uninfected strain (R. L. Miller, unpublished results and Figure 2; T3 probe lanes 1 vs. 4,). The first species of RNA (V) has been previously identified as the 7 kb dsRNA viral genome of GLV (1). The second species identified (SS) migrated at an apparent

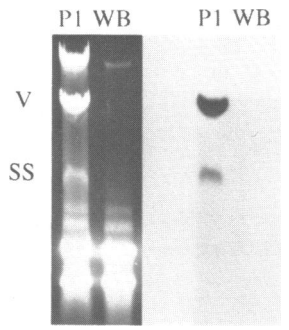

Figure 3. Northern Analysis of Portland I Total RNA Using a Probe from the 3' End of the GLV dsRNA.

Portland I and WB total RNA (10 µg/lane) were electrophoresed in a 0.8% agarose gel and the nucleic acid was transferred to nitrocellulose, as described in the Methods. A 3' end RNA probe (16,000 cpm) was prepared (as described in the Methods) and was hybridized to the filter, followed by autoradiography. V and SS are viral dsRNA and ssRNA respectively.
a) Ethidium bromide stained gel.
b) Autoradiogram of the nitrocellulose filter.

size of 6.8 kb using single-stranded markers. When total RNA from Portland I was denatured with 10% formamide (Figure 2; T3 probe, lane 2) or 40% formamide (Figure 2; T3 probe, lane 3), the denatured V comigrated with SS by agarose gel electrophoresis. These data suggest that SS is a single-stranded RNA copy of GLV. The following experiments were designed to demonstrate whether or not SS is a full length copy of one of the strands in the GLV dsRNA genome (V).

Total RNA from Portland I and WB strains of *G. lamblia* was fractionated by agarose gel electrophoresis before and after denaturation and then transferred to nitrocellulose (Figure 2; T7, T3 probes). The filter was probed separately with T7 and T3 RNA polymerase transcripts from the clone pG30 (Figure 1). Only the T7 transcript annealed to the region of the filter corresponding to SS (Figure 2; T7 probe, lane E vs. T3 probe, lane A), whereas both T3 and T7 transcripts annealed to the region of the filter corresponding to V and denatured V (Figure 2; T7 probe, lane E - G and T3 probe, lane A - C). These data suggest that SS is a single strand, not denatured dsRNA genome (V). In addition, hybridizations using kinased (^{32}P)DNA oligonucleotide probes based on the sequence of pG30 (see Figure 1) gave similar results (data not shown). Figure 2 shows that the T3 RNA polymerase transcript of pG30 also hybridizes to the *G. lamblia* small subunit (SSU) rRNA. However, the SSU rRNA (15) and pG30

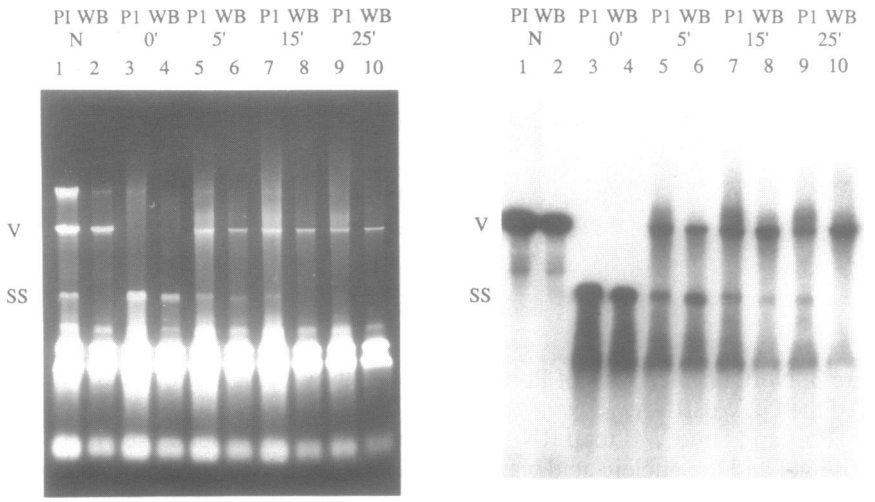

Figure 4. Denaturation-Renaturation of GLV dsRNA in Portland I and WB Total RNA.

Total RNA of WB (lanes 2, 4, 6, 8, 10) and Portland I (lanes 1, 3, 5, 7, 9) (100 µg/ml) and 3,000 cpm of (^{32}P) pCp-labeled V were combined in 0.17 X TBE, 10% formamide in a total volume of 30 µl. 100 ng of V was added to the WB mixtures so that all of the samples would have the same amount of V. The samples in lanes 3 - 10 were heated at 90°C for 5 minutes then quenched on ice for 1 minute to denature the dsRNA. After denaturation, 1 M NaCl (3.5 µl) was added to all of the samples. The samples in lanes 3 - 10 were renatured for the times indicated in the Figure by incubation at 63°C. The renaturation reactions are quenched by freezing the sample at -70°C. The samples in lanes 1 and 2 were control samples showing V without denaturation-renaturation treatment. Ficoll loading dye (5 µl) was added to all of the samples and the samples were electrophoresed in a 0.8% agarose gel in TBE and 5 mg/ml ethidium bromide. After photography of the ethidium bromide stained gel, the gel was dried and set to expose film for 24 hours at -70°C. V is the viral dsRNA and SS is the viral ssRNA.
a) Ethidium bromide stained gel.
b) Autoradiograph of the dried gel.

show no sequence homology, and the DNA oligonucleotides did not hybridize with the SSU rRNA.

To provide further evidence that SS is a full length copy of one of the strands of V, a Northern blot of Portland I total RNA was hybridized with an RNA probe that was within 170 nucleotides of the 3' ends of V (Figure 3). This probe was generated by labeling the 3' ends of V with (^{32}P)pCp and then partially digesting the labeled viral genome with RNAse U2 as described in the

Methods. Both V and SS hybridize with the probe suggesting that SS contains sequences complementary to the 170 nucleotides at the 3' end of V. It seems likely that these complementary sequences would be at the 5' end of SS, though these data do not prove that. Unfortunately, we could not efficiently kinase the 5' end of V and therefore the 3' end of SS could not be probed in an analogous experiment. Since the kinase reaction with V is not enhanced by either a preliminary phosphatase treatment or the inclusion of ADP in the reaction mixture (data not shown) it seems unlikely that the 5' end of V is phosphorylated. The low efficiency of the kinase reaction may be due to a 5' cap, steric hinderance from some secondary structure, or a recessed 5' end. Experiments to test this hypothesis are underway.

In order to further characterize the homology between SS and V, an experiment was carried out to determine whether SS could exchange with a corresponding strand of V. If SS is a full length copy of one of the strands of V, then SS should displace some of the identical strand of V when V is denatured and renatured in the presence of SS. Attempts to do this experiment with labeled SS were unsuccessful because we were unable to efficiently end label SS (data not shown). As an alternative, V was labeled at the 3' end with (^{32}P)pCp. This labeled V was then mixed with either Portland I total RNA or WB total RNA. Unlabeled V was added to the WB mixture to the concentration present in the Portland I extract. In the final conditions the primary difference between the Portland I and the WB mixtures was the presence of SS in the Portland I mixture. The two mixtures were denatured with formamide and then renatured (Figure 4 a,b). The renaturation of V was complete in both the Portland I and the WB extracts after 25 minutes. However, the Portland I extract showed substantially more radioactivity comigrating with SS than the WB extract at both the 15 and 25 minute renaturation time points. These data suggest that SS is displacing the corresponding strand of labeled RNA from the dsRNA genome of the GLV. In a second experiment, the kinetics of the reannealing process were further examined to show that SS was a copy of one strand of V. In this experiment, the concentration of V was low compared to the preceding experiment but SS was at a similar concentration. Therefore, during the time of the experiment, the denatured V strands should not reanneal unless SS was present in the mixture to drive the reannealing process. First, V was removed from the Portland I total RNA by a fractionating ethanol precipitation described in the Methods. This procedure generates a solution which primarily contains the ribosomal RNA's and SS. A solution of WB total RNA was prepared with the same method. The denaturation-renaturation experiment was performed with a lower

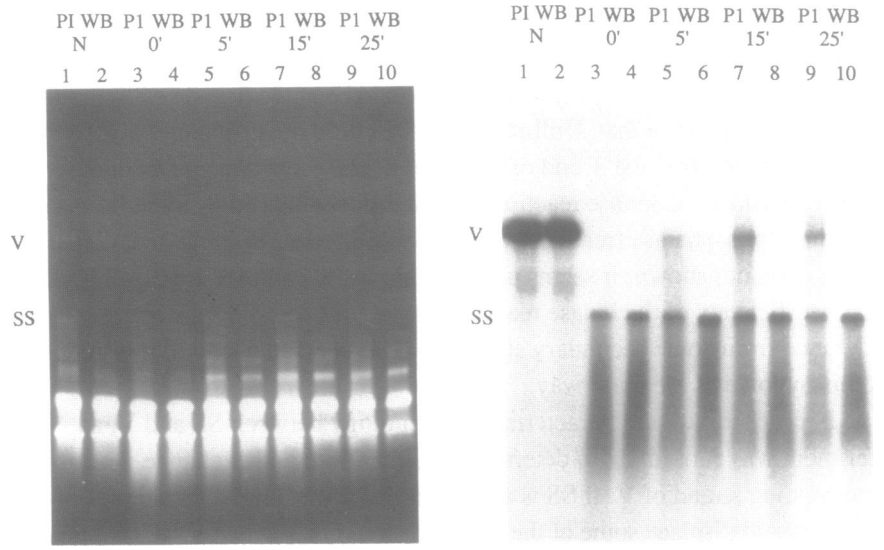

Figure 5. Denaturation-Renaturation of GLV dsRNA in Portland I "dsRNA Depleted" Total RNA and WB Total RNA.

V depleted total RNA (1.5 μg) from Portland I (lanes 1, 3, 5, 7,9) or analogously treated total RNA from WB (lanes 2, 4, 6, 8, 10) were combined with (^{32}P) labeled GLV dsRNA (3,000 cpm, 30 ng) in 0.17 X TBE and 10% formamide in a total volume of 30 μl. The samples in lanes 3 - 10 were heated at 90°C for 5 minutes then quenched on ice for 1 minute to denature the dsRNA. After denaturation, 1 M NaCl (3.5 μl) was added to all of the samples. The samples in lanes 3 - 10 were renatured for the times indicated in the Figure by incubation at 63°C. The renaturation reactions were quenched by freezing the sample at -70°C. The samples in lanes 1 and 2 were control samples showing untreated GLV dsRNA. Ficoll loading dye (5 μl) was added to all of the samples. The samples were electrophoresed in a 0.8% agarose gel in TBE and 5 mg/ml ethidium bromide. After photography of the ethidium bromide stained gel, the gel was dried and set to expose film for 24 hours at -70°C. V is the viral dsRNA and SS is the viral ssRNA.
a) Ethidium bromide stained gel.
b) Autoradiograph of the dried gel.

concentration (higher specific activity) of 3' labeled V (Figure 5). Under the conditions of this experiment the denatured V in the WB RNA mixture does not reanneal to a significant extent. However, in the RNA mixtures containing SS, reannealing occurs quite facilely. Therefore, it appears that the unlabeled SS drives the annealing process.

The data presented thus far strongly suggest that SS is a full length copy of one of the strands of V. Therefore, it seems likely that SS might be a viral

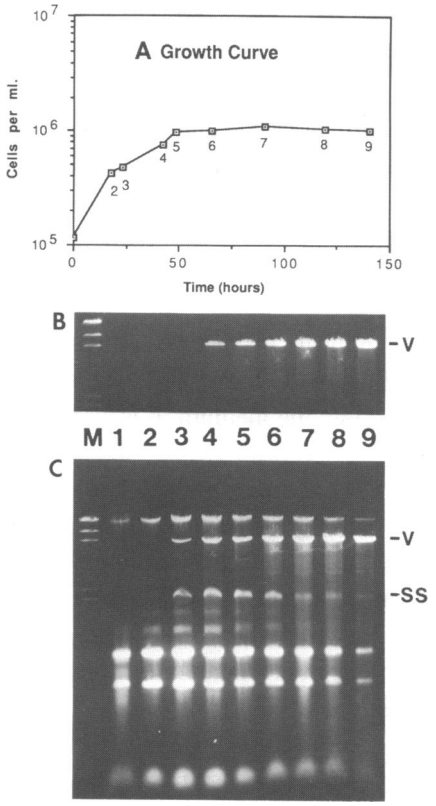

Figure 6. Presence of the ssRNA and dsRNA in Total RNA from Cells after Infection with GLV.

Panel A. *G. lamblia* growth curve. Samples were inoculated with approximately 10^5 cells and time points were taken at the indicated times. At each time point, the cell concentration was determined, viral RNA was extracted from the medium and total RNA was extracted from the cells. Time points are 2 = 18 h., 3 = 23 h., 4 = 42 h., 5 = 49 h., 6 = 66 h., 7 = 91 h., 8 = 119 h., and 9 = 141 h.

Panel B. Viral RNA extracted from the medium at each time point. Equal amounts of medium (3 ml.) were used for each time point. M = λ DNA cut with Hind III. V = viral dsRNA.

Panel C. Total RNA extracted from cells at each time point. Total RNA from approximately 4 x 10^6 cells was loaded in each lane. The bands above the viral RNA are residual DNA. The lower two bands represent the rRNAs. Bands which are visible between the ssRNA and the rRNAs are rRNA/rRNA complexes. Lane 1 represents total RNA from uninfected cells. V = viral dsRNA, SS = viral single-stranded RNA.

replication intermediate or mRNA which should be most prevalent during the stages of maximal growth of the virus. If, however, the SS is an artifact of purification or the product of a side reaction, then it should be present in the cell at the same concentrations during all phases of viral growth. To distinguish between these two possibilities, we infected strain WB with GLV and followed the growth of the cells, the amount of viral RNAs inside of the cells, and the amount of virus outside of the cells(Figure 6). Figure 6 shows that the cells grew normally through log phase (time points 2 and 3, late log phase (time points 4 and 5), stationary phase (time points 6 and 7) and late stationary phase (time points 8 and 9). It has been shown previously (4) that the growth of the cells is not affected by infection with GLV (M.O.I. of 1,000). In Panel B, we have monitored the virus excreted into the medium. It is clear that virus is not excreted until approximately 24 hours after infection and that the virus in the medium reaches a constant level as the cells reach late stationary phase. It is unlikely that the lack of virus in the medium before 24 hours is due to the lower number of cells because a comparison of the measurement at time point 4 with the measurement at time point 3 shows a modest increase in cell number (two fold) but large increase (at least 10 fold) in virus in the medium. These data suggest that the amount of virus in the medium is more a function of the viral replication state than the cell growth phase. In Panel C, we have monitored the viral RNAs inside the cells. The viral RNA (V in Figure 6) is detectable within 18 hours after infection and reaches a plateau as the cells remain in stationary phase. Comparing the level of V in the medium with the level of V in the cells, it is obvious that V emerges and plateaus sooner within the cells than outside of the cells. The SS behaves as one would expect of a replication intermediate or mRNA. The SS is most prevalent at times when the production of V is maximal (lanes 3, 4, and 5). At later time points (lanes 6 to 9) viral RNA production has leveled off and the amount of SS has been reduced dramatically.

Since the time course suggests that SS may be the viral mRNA, it was important to determine if SS was polyadenylated. Portland I total RNA was separated into polyA+ RNA and polyA- RNA by oligo-dT column chromatography. The separated polyA+ and polyA- nucleic acid mixtures were then chromatographed by agarose gel electrophoresis. The nucleic acids were transferred to nitrocellulose and hybridized to the oligonucleotides shown in Figure 1, as described in the methods. SS was observed to be enriched in the polyA- fraction (data not shown).

DISCUSSION

There are two major species of RNA present in GLV infected strains of *G. lamblia* (e.g. Portland I) and not in uninfected strains (e.g. WB). The first species (V) is the GLV dsRNA genome (1). The second species (SS) comigrates with denatured V on agarose gel electrophoresis, suggesting that SS is close to the same nucleotide length as V but is single-stranded in nature. Northern analysis of the SS shows that it is capable of annealing to the transcript from only one strand of the clone pG30. In addition, RNA probes derived from within 170 nucleotides of the 3' end of V anneal to SS suggesting that it has complementary sequences. t seems likely that these complementary sequences are at the SS 5' end. SS also corresponds to the "sense" strand that generated the only open reading frame in pG30. In addition to the Northern results, SS can exchange with a strand from the dsRNA in denaturation-renaturation experiments. All of these data suggest that SS is a full length copy of one of the strands of the GLV dsRNA genome.

The T7 probe also showed hybridization to the SSU rRNA but since this hybridization occurs in both the infected and uninfected strains of the parasite, it is clear that the hybridization is not due to some viral nucleic acid species that fortuitously comigrates with the SSU rRNA. Though the sequences of pG30 and the SSU rRNA show no obvious homology and the DNA oligos do not hybridize, the fact that the probe hybridizes with only one of the rRNA subunits argues that the interaction is specific. We are presently investigating the nature of the interaction between pG30 and the SSU rRNA since it may play a role in the viral replication process.

The levels of SS observed in the time course of GLV infection of the WB strain are consistent with its role as an mRNA or a replicative intermediate. The SS appears earlier in the infection than the mature virus in the medium. Also, the ratio of SS to V is higher in the early and middle stages of viral production than in the later stages suggesting that large amounts of SS are produced early as template for the final viral genome and as message for the viral coat protein synthesis. As far as we can determine, SS is not polyadenylated although some *G. lamblia* RNA is polyadenylated (unpublished observation). However, these data do not completely exclude SS from being the virus message strand. In fact, since the data suggest that SS is a full length copy of one of the strands of V, the time course data imply that SS is a viral replication intermediate and a viral mRNA. Further pulse/chase experiments and the *in vitro* translation of SS will be necessary to prove this hypothesis. In an apparently analogous system, yeast cells

infected with the *S. cerevesiae* killer virus also contain full length single-stranded copies of the dsRNA genome, that have been regarded as replicative intermediates (16). In addition, both the single-strand species and denatured double-strande ' yeast killer virus RNA have been successfully translated *in vitro* to produce the major coat protein of the virus (17,18).

Presently, there is no genetic vector capable of transforming *G. lamblia*, and the characteristics of GLV make it an attractive target for development as a genetic vector for *G. lamblia*. Therefore, a full length clone of the virus must be obtained. Recently (19), a large cDNA clone (6.3 kb) of a dsRNA virus genome from bacteriophage ϕ 6 has been obtained by utilizing a full length ssRNA copy of the virus genome as a template for reverse transcriptase. This paper describes a single-stranded species of RNA which appears to be a full length copy of one of the strands of the GLV dsRNA genome. Utilization of this ssRNA species as a template for reverse transcriptase should expedite obtaining a full length clone of GLV.

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ABBREVIATIONS

TBE = Tris Borate EDTA (7), EDTA = Ethylenediamine tetraacetic acid, DEPC = Diethylpyrocarbonate, pCp = cytidine 3', 5', bisphosphate, ATP = Adenosine triphosphate, SDS = sodium dodecyl sulfate, DMSO = Dimethylsulfoxide. SSPE, SSC, PBS, and Denhardts were prepared as described (7). SS = GLV ssRNA, V = GLV dsRNA, GLV = *Giardia lamblia* virus.

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REFERENCES

1. Wang, A. L. and Wang, C. C. (1986) Mol. Biochem. Parasitol., 21, 269 - 276.
2. Pickering, L. K. and Engelkirk, P. G. (1988) New Topics in Infectious Disease, 35, 565 - 577.
3. Miller, R. L., Wang, A. L., and Wang, C. C. (1988) Exp. Parasitol., 66, 18 - 123.
4. Miller, R. L., Wang, A. L., and Wang, C. C. (1988) Mol. Biochem. Parasitol., 28, 189 - 196.

5. Wickner, R. B. (1986) Ann. Rev. Biochem., 55, 373 - 395.
6. Buck, K. W., Ackermann, H. W., Bozarth, R. T., Bruenn, J. A., Koltin, Y., Rawlinson, C. J., Ushiyama, R., and Wood, H. A. (1984) Intervirology, 22, 17 -23.
7. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
8. Scherrer, K. and Darnell, J. E. (1962) Biochem. Biophys. Res. Comm. 7, 486 - 490.
9. Englund, T. E., Bruce, A. G., and Uhlenbeck, O. C. (1980) Meth. Enzymol. 65, 65 - 74.
10. Wang, A. L. and Wang, C. C. (1985) J. Biol. Chem. 260, 3697 - 3702.
11. de Lange, T., Liu, A. Y. C., van der Ploeg, L., Borst, P., Tromp, M. C., and van Boom, J. H. (1983) Cell 34, 891 - 900.
12. Hatori, M. and Sakaki, Y. (1986) Anal. Biochem., 152, 232 - 238.
13. Zagursky, R. J., Baumeister, K., Lomax, N., and Berman, M. L. (1985) Gene Anal. Techn., 2 89 - 94.
14. Mills, D. R. and Kramer, F. R. (1879) Proc. Natl. Acad. Sci. (USA), 76, 2232 - 2235.
15. Sogin, M. L., Gunderson, J. H., Elwood, H. J., Alonso, R. A., and Peattie, D. A. (1989) Science, 243, 75 - 77.
16. Esteban, R., Fujimura, T., and Wickner, R. B. (1988) Proc. Natl. Acad. Sci. (USA), 85, 4411 - 4415.
17. Hopper, J. E., Bostian, K. A., Rowe, L. B., and Tipper, D. J. (1977) J. Biol. Chem., 252, 9010 - 9017.
18. Bruenn, J., Bobeck, L., Brenan, V., and Held, W. (1980) Nucleic Acids Res., 8, 2985 - 2997.
19. Mindich, L., Newhauser, I., Gottlieb, P., Romantschuk, M., Carton, J., Frucht, S., Straussman, J., Bamford, D. H., Kalkkinen, N. (1989) J. Virol., 62, 1180 - 1185.