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Characterisation of the subtelomeric regions of *Giardia lamblia* genome isolate WBC6

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Abstract

Giardia trophozoites are polyploid and have five chromosomes. The chromosome homologues demonstrate considerable size heterogeneity due to variation in the subtelomeric regions. We used clones from the genome project with telomeric sequence at one end to identify six subtelomeric regions in addition to previously identified subtelomeric regions, to study the telomeric arrangement of the chromosomes. The subtelomeric regions included two retroposons, one retroposon pseudogene, and two *vsp* genes, in addition to the previously identified subtelomeric regions that include ribosomal DNA repeats. The presence of *vsp* genes in a subtelomeric region suggests that telomeric rearrangements may contribute to the generation of *vsp* diversity. These studies of the subtelomeric regions of *Giardia* may contribute to our understanding of the factors that maintain stability, while allowing diversity in chromosome structure.

Keywords: *Giardia lamblia*; Telomere; Subtelomeric region; *vsp*; Retroposon

1. Introduction

Giardia lamblia is a flagellated unicellular eukaryotic protozoan parasite that is a common cause of diarrheal disease worldwide. The life cycle of *Giardia* consists of two stages, the infectious cyst and the vegetative trophozoite. Infection occurs when the host ingests the environmentally stable cyst via a contaminated water or food source. After passage through the stomach, the cysts excyst into trophozoites, which multiply in the proximal small intestine. A few trophozoites encyst in the jejunum after exposure to biliary fluid and are excreted via the feces, thus completing the life cycle.

Giardia trophozoites have two nuclei that are both transcriptionally active (Kabnick and Peattie, 1990) and replicate at approximately the same time (Wiesehahn et al., 1984). Trophozoites are polyploid and have five chromosomes ranging in size from approximately 1.6 to 3.8 Mb, although some isolates have size variants of chromosome 1 as small as 1.1 Mb (Adam et al., 1988). This adds up to a haploid genome size of approximately 10–12 Mb, a value which is supported by the results of the genome project (www.mbl.edu/Giardia). Each nucleus has at least one copy of each chromosome (Yu et al., 2002) and the two nuclei have approximately equal quantities of DNA. Therefore, since trophozoites have an estimated ploidy of four (Bernander et al., 2001), it is likely that each nucleus has a ploidy of approximately two. However, the exact number of copies of each chromosome in each nucleus may vary somewhat, as suggested by a cytogenetic evaluation in which the number of chromosomes per nucleus varied between nine and 11 (Tumova et al., 2006), leaving open the possibility of a small degree of aneuploidy.

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Previous studies of the *Giardia* chromosomes have demonstrated substantial size variation of chromosome 1 (Adam et al., 1988, 1991; Adam, 1992; Le Blancq et al., 1991a, 1992, 1991b; Hou et al., 1995). These analyses of chromosome 1 demonstrated a core region that was highly similar for all copies of the chromosome and substantial variation in the subtelomeric regions (STRs). Ribosomal DNA (rDNA) repeats were found adjacent to the TAGGG telomeric repeat at one end of chromosome 1 and accounted for a substantial portion of the size heterogeneity for the different chromosome 1 homologues (Adam, 1992; Hou et al., 1995). Different *Giardia* isolates demonstrate substantial differences in the chromosomal locations of the rDNA repeats (Adam et al., 1991), even for different cloned lines of the same isolate (Adam and Prabhu, unpublished observations). In addition to being present as repeating units, the rRNA genes may be present in subtelomeric regions as rearranged or incomplete genes (Upcroft et al., 2005).

In addition to rDNA, other STRs have been reported. A chromosome 1 STR (p4e) was cloned on the basis of having the telomeric repeat at one end (Hou et al., 1995). Clones have been identified that have telomeric repeats at one end and a retroposon sequence at the other (Arkhipova and Morrison, 2001). Three different retroposons were identified, GilM, GilT and GilD. All three belong to long interspersed nuclear element (LINE)-like families. GilM and GilT have intact reverse transcriptase open reading frames (ORFs) and are candidates for functional genes, but whether they are expressed has not yet been published. On the other hand, GilD has multiple deletions, stop codons and frameshifts, so it is presumably a pseudogene.

A telomeric *vsp* gene was identified in the same study. In addition, previous mapping studies had shown that the *vsp*s, CRP65 and CRP136, hybridised to *Xba*I fragments that also contained the telomeric repeat sequence (Upcroft et al., 1997; Chen et al., 1995, 1996), but in these mapping studies, rDNA and ankyrin were found between the *vsp* and the telomeric repeat. It is of interest to know if the *vsp*s are telomeric, in view of the fact that surface antigen genes of other protists may be expressed from subtelomeric locations.

In the current study, we have used the telomeric clones from the genome project to determine the chromosomal organisation of the STRs of the genome isolate WBC6.

2. Materials and methods

2.1. Growth and cloning of Giardia isolates

The genome isolate, WB clone C6 (ATCC #50803), was the major isolate used for this study (McArthur et al., 2000; Gillin et al., 1990). Trophozoites were grown in modified TYI-S-33 medium (Keister, 1983). Cloning was performed by limiting dilution as previously described (Nash et al., 1988).

2.2. Identification and directed sequencing of telomeric clones

Telomeric clones that were sequenced as part of the *Giardia* genome project were chosen for further analysis. The plasmid clones used for this project were obtained from small insert plasmid libraries with inserts generated by partial enzyme digestion or by random shearing of the DNA (*Giardia lamblia* Genome Project website: www.mbl.edu/Giardia). Since chromosome-internal telomeric sequences also occur, we surveyed the assembled sequence for the presence of TAGGG telomeric repeats. The longest chromosome-internal telomeric sequence was 18 bp. Therefore, we limited our analysis to clones with at least four copies of the telomeric repeat at one end of the clone. Previous studies have reported the telomeric location of rDNA. Therefore, we limited our analysis to clones with a sequence other than rDNA at the nontelomeric end.

For each subtelomeric region, we chose at least one clone for complete bidirectional sequencing which was obtained by primer walking. However, for some clones, the read from the telomeric end did not go beyond the telomeric repeat. For those clones, we were only able to sequence a single direction for a small portion of the telomere transition region adjacent to the telomeric repeats. When more than one clone was available for a subtelomeric region, we obtained a complete single pass sequence for the additional clone(s). The GenBank accession numbers are shown in Table 1.

2.3. Pulsed field gel electrophoresis (PFGE)

Pulsed field gel electrophoresis (PFGE) of total *Giardia* DNA was performed as described (Adam et al., 1988) using OFAGE (orthogonal field alternation gel electrophoresis) with 1% Seakem GTG agarose (Cambrex Bioscience, Rockland, ME) and switching intervals of 11 min at 80 V for 2 days, 25 min at 65 V for 2 days and 60 min at 50 V for 3 days. When Seakem Gold agarose (Cambrex Bioscience, Rockland, ME) was used, 0.8% gels were run with switching intervals of 6 min at 80 V for 1 day, 11 min at 65 V for 1 day and 25 min at 50 V for 3 days. The separated DNA was either transferred to Nytran (Schleicher and Schuell, Keene, NH) by alkaline transfer, or individual chromosomal bands were removed for restriction enzyme digestion. The individual chromosomes were cut into blocks and enzymatically digested following equilibration in the appropriate buffer. After equilibration, 200 µl of fresh buffer along with 20 U of enzyme was added and the blocks were incubated overnight at 4 °C. The tubes were then brought to the appropriate digestion temperature for 2 h, after which 20 U of enzyme was added for an additional 2 h of digestion. The digested blocks were separated by CHEF (Contour-clamped homogeneous electric field) in 1% GTG agarose for 22 h at 120 V with a