

Analysis of a *Giardia lamblia* rRNA encoding telomere with $[TAGGG]_n$ as the telomere repeat

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The ribosomal RNA (rRNA) genes of *Giardia lamblia* are organized in tandem arrays of a 5.6 kb repeat (1, 2). Genomic DNA digested with the restriction endonuclease XbaI, which does not cut within the rRNA repeat, showed a fuzzy rRNA hybridizing band of 6.5 to 7.5 kb (Figure 1A). We examined the potential telomeric location of these rRNA-containing fragments by digesting genomic DNA with BAL 31 followed by digestion with XbaI (Figure 1A). BAL 31 digestion resulted in the disappearance of the 7 kb smear and the appearance of a homogeneous band that was progressively shortened (15 bp/min). A band at 10 kb also showed BAL 31 sensitivity. The high molecular weight DNA encoding rRNA genes readily distributes into a smear upon continued BAL 31 digestion, indicative of other telomerically located rRNA genes (3). Shortening of fragments hybridizing with a β-tubulin control probe could not be detected (Figure 1C). We concluded that we had identified telomeres containing rRNA coding sequences.

We cloned the 7 kb telomeric XbaI restriction fragment by subjecting genomic DNA to a brief BAL 31 digestion, then to digestion by XbaI, size fractionation and ligation into a plasmid. The physical maps of the telomere clone and the genomic DNA fraction were identical (as determined by Southern analysis). The telomere clone (pGC1) contained a truncated rRNA gene repeat unit whose 5′ and 3′ ends occurred in the coding region of large subunit rRNA genes (Figure 2): non-rRNA sequence precedes the 5′ end and a tandem array of the repeat $[TAGGG]_n$ follows the 3′ end. The three G residues at the 3′ junction could derive from either the rRNA sequence or from the first TAGGG repeat (Figure 2). The telomeric nature of the tandem array of TAGGG repeats and the lack of chromosome internal $[TAGGG]_n$ was confirmed in BAL 31 treated total genomic DNA (Figure 1B). This experiment also indicated that the average length of the telomere repeats did not exceed 1 kb since all $[TAGGG]_n$ hybridisation was eliminated after BAL 31 digestion of 1 kb of DNA. The telomere repeat was not degenerate, but we saw two examples of a single TAAGG pentanucleotide repeat within the array of >100 telomere repeats. [TAGGG] is the shortest invariant telomere repeat unit identified thus far (4).

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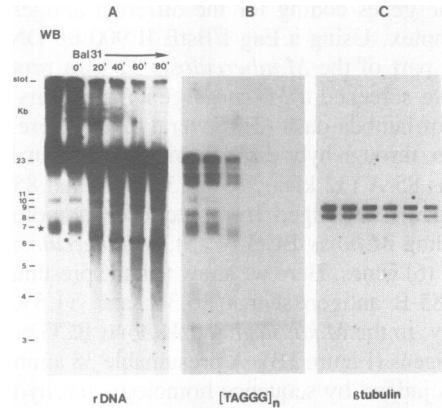

Figure 1. BAL 31 sensitivity of DNA containing rRNA genes in *G. lamblia*. High molecular weight DNA from the *G.lamblia* strain WB (ATCC 30957) was subjected to BAL 31 exonuclease digestion for 0, 20, 40, 60, 80 min and then digested with XbaI and run out into a 0.5% agarose gel. The gel was blotted and hybridized with a rRNA probe from pGRPI (1) (Panel A). Lane 1 contains non-BAL 31 treated DNA. The filter was re-hybridised with a β-tubulin probe (panel C) and a telomere repeat probe $[TAGGG]_n$ (panel B).

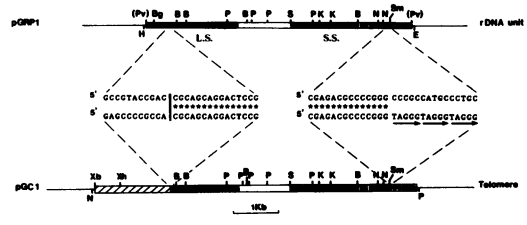

Figure 2. Physical map of the telomeric clone pGC1. The telomere clone, pGC1, is shown with the cloned rRNA repeat unit, pGRPI, aligned above (1). Blocks represent rRNA sequences: open boxes = rRNA spacer regions, LS = large subunit gene, SS = small subunit gene. The hatched region is non-rRNA flanking sequence. The boxed arrowheads indicate the telomere repeat tandem array. The sequences of the junction regions and the corresponding region in the cloned rRNA repeat unit are shown between the two maps, with homology indicated by asterisks. Plasmid sequences are shown as thin lines. B, BamHI; Bg, BglII; E, EcoRI; H, HindIII; K, KpnI; P, PstI; Pv, PvuII; N, NotI; S, SphI; Sm, SmaI; Xb, XbaI; Xh, XhoI. All sites for each enzyme are shown except for SmaI.