Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal for Parasitology 35 (2005) 1435–1443
www.elsevier.com/locate/ijpara

Genomic organization of telomeric and subtelomeric sequences of *Leishmania* (*Leishmania*) *amazonensis*

F.F. Conte, M.I.N. Cano*

Departamento de Patologia Clínica, Núcleo de Medicina e Cirurgia Experimental, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), CP 6109, 13083-970, Campinas, SP, Brazil

Received 28 February 2005; received in revised form 20 May 2005; accepted 24 May 2005

Abstract

Telomeres are DNA-protein complexes that protect linear chromosomes from degradation and fusions. Telomeric DNA is repetitive and G-rich, and protrudes towards the end of the chromosomes as 3′G-overhangs. In *Leishmania* spp., sequences adjacent to telomeres comprise the *Leishmania* conserved telomere associated sequences (LCTAS) that are around 100 bp long and contain two conserved sequence elements (CSB1 and CSB2), in addition to non-conserved sequences. The aim of this work was to study the genomic organization of *Leishmania* (*Leishmania*) *amazonensis* telomeric/subtelomeric sequences. *Leishmania amazonensis* chromosomes were separated in a single Pulsed Field Gel Electrophoresis (PFGE) gel as 25 ethidium bromide-stained bands. All of the bands hybridized with the telomeric probe $(5'\text{-TTAGGG-}3')_3$ and with probes generated from the conserved subtelomeric elements (CSB1, CSB2). Terminal restriction fragments (TRF) of *L. amazonensis* chromosomes were analyzed by hybridizing restriction digested genomic DNA and chromosomal DNA separated in 2D-PFGE with the telomeric probe. The *L. amazonensis* TRF was estimated to be ~3.3 kb long and the telomeres were polymorphic and ranged in size from 0.2 to 1.0 kb. *Afa* I restriction sites within the conserved CSB1 elements released the telomeres from the rest of the chromosome. *Bal* 31-sensitive analysis confirmed the presence of terminal *Afa* I restriction sites and served to differentiate telomeric fragments from interstitial internal sequences. The size of the *L. amazonensis* 3′ G-overhang was estimated by non-denaturing Southern blotting to be ~12 nt long. Using similar approaches, the subtelomeric domains CSB1 and CSB2 were found to be present in a low copy number compared to telomeres and were organized in blocks of 0.3–1.5 kb flanked by *Hinf* I and *Hae* III restriction sites. A model for the organization of *L. amazonensis* chromosomal ends is provided.

Keywords: *Leishmania amazonensis*; *Leishmania* conserved telomere associated sequences; Pulsed field gel electrophoresis; Subtelomeric region; Telomeres; Terminal restriction fragments

1. Introduction

Telomeres are essential for genome stability in all eukaryotes, and this makes them very attractive targets for the development of anti-parasitic drugs. These terminal structures serve as functional complexes that protect chromosomal ends by preventing terminal fusions and degradation (Blackburn, 2000; 2001). Telomeres consist of an array of telomeric repeats associated with proteins and extend towards the ends of chromosomes as G-rich single-strand protrusions known as 3′ G-overhangs. The latter structures are formed during the replication of the lagging strand of DNA (Henderson and Blackburn, 1993) and can form T loops (telomere loops) in mammals, ciliates and trypanosomes (Griffith et al., 1999; Murti and Prescott, 1999; Muñoz-Jordan et al., 2001). In almost all organisms, telomeres are kept within a size range that is characteristic of each organism or cell type. Telomeres are not fully replicated by DNA polymerase and gradually shorten, unless they are actively maintained by telomerase (Autexier and Greider, 1996).

The chromosomal termini of *Leishmania* spp. contain conserved 5′-TTAGGG-3′ telomeric repeats (Blackburn and Challoner, 1984; Fu and Barker, 1998; Cano, 2001), the organization of which depends on the parasite species

* Corresponding author. Tel.: +55 19 37887390; fax: +55 19 38777414.
E-mail address: micano@unicamp.br (M.I.N. Cano).

0020-7519/$30.00 © 2005 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijpara.2005.05.011

(Fu and Barker, 1998; Chiurillo et al., 2000). For example, the telomeres of *Leishmania braziliensis* have a non-conserved telomeric repeat 5′-CCTAACCCGTGGA-3′ (Fu and Barker, 1998; Fu et al., 1998). Adjacent to the telomeres are subtelomeric regions that contain numerous polymorphic repeats (Ravel et al., 1995; Pryde et al., 1997; Fu and Barker, 1998; Fu et al., 1998; Chiurillo et al., 2000), showing a high degree of polymorphism. Homologs of *Leishmania major* chromosome 1 have an ~20 kb length difference in the subtelomeric region (Myler et al., 1999; 2000). There is evidence that recombination events among subtelomeric sequences can lead to chromosomal polymorphism and genomic rearrangements (Lanzer et al., 1995; Myler et al., 1999).

The subtelomeric regions of almost all species of *Leishmania* studied so far (Fu and Barker, 1998) are referred to as *Leishmania* conserved telomere associated sequences (LCTAS) that occur as ~100 bp sequences composed of two conserved elements, CSB1 (conserved sequence block 1) and CSB2 (conserved sequence block 2), separated one from the other by 1–51 bp. However, LCTAS differ greatly in their organization, depending on the chromosome or species (Fu and Barker, 1998; Fu et al., 1998; Myler et al., 2000; Chiurillo et al., 2000). If LCTAS play a role in chromosomal segregation or as binding sites for telomeric proteins should be investigated (Fu and Barker, 1998; Stiles et al., 1999).

The aim of this work was to study the chromosomal organization of the telomeric and subtelomeric regions of *Leishmania (Leishmania) amazonensis*. Using several approaches, we estimated the terminal restriction fragment (TRF) length of *L. amazonensis* chromosomes and found that the digestion of DNA with *Afa* I physically separates very polymorphic telomeres from the rest of the chromosomes. *Bal* 31-sensitive analysis was used to confirm these findings and to differentiate telomeric fragments from interstitial internal sequences. We also estimated the size of the *L. amazonensis* 3′ G-overhang using non-denaturing Southern blotting and found that the subtelomeric domains CSB1 and CSB2 were present in a low copy number compared to telomeres and were organized in blocks flanked by *Hinf* I and *Hae* III restriction sites.

2. Materials and methods

2.1. Parasite cultures and extraction of genomic DNA

Promastigotes of *L. amazonensis*, strain MHOM/BR/73/M2269, were maintained in exponential phase at 27 °C in Schneider's *Drosophila* medium (Sigma), pH 6.5, supplemented with 5% FCS (Cultilab) and 1 X antibiotic/antimycotic (Life Technologies, Gibco-BRL). Total genomic DNA of *L. amazonensis* was prepared according to (Cotrim et al. (1990)).

Table 1
Telomeric and subtelomeric oligonucleotides used in this study

Oligonucleotides	Sequence
Tel	5′-TTAGGGTTAGGGTTAGGG-3′
CSB1 F	5′-GTACAGTGTACAGTGTACAGT-3′
CSB2 R	5′-GGAGAGGGTGTGGAGAGGGTGT-3′
Tel 12 C	5′-CCTAACCCTAAC-3′

2.2. Southern and chromosomal blotting

Southern blotting, hybridization and washing were done using standard procedures (Chiurillo et al., 1999; Sambrook and Russel, 2001). An 18-mer oligonucleotide (Tel) containing three tandem telomeric repeats of the hexamer 5′-GTTAGG-3′ was used as a probe. For hybridization, telomeric and subtelomeric oligonucleotides (see Table 1) were 5′-end-labeled with $\gamma[^{32}P]$ATP using T4 polynucleotide kinase (Amersham Biosciences), according to the manufacturer's instructions. The hybridization conditions were the same as described by Chiurillo et al. (1999), except that *Escherichia coli* tRNA was not used in the hybridization solution and the blots were washed twice for 10 min at room temperature.

2.3. Estimation of the terminal restriction fragments (TRF) of L. amazonensis chromosomes

The TRF of *L. amazonensis* were estimated as described by Levy et al. (1992). Terminal restriction fragments correspond to the terminal fragments of chromosomes, including telomeres and part of the subtelomeric region. To estimate the TRF, it was necessary to calculate the signal intensity of each hybridized DNA fragment using Scion Image processing and analysis software (www.scioncorp.com). The mean length of the TRFs was calculated using the formula:

$$L = \Sigma(ODi \times Li)/\Sigma(ODi)$$

where ODi and Li are, respectively, the signal intensity and the length of the TRF at position i of the gel image. The method consists in first to scan the autoradiograms and them to divide the scanned image into a grid consisting of the number of columns in the gel (or sample lanes) divided in multiple boxes (at least $n = 30$) of the same size. Thus, 'i' denotes box number from 1 to 'n', where 'n' is the number of grid boxes in a column (gel lane).

2.4. Non-denaturing Southern blotting of the 3′G-overhang protrusions at the terminus of L. amazonensis chromosomes

This experiment was based on the protocols described by Dionne and Wellinger (1996). Genomic DNA (10 μg) was digested with 10 U of *Hae* III, *Afa* I and *Hinf* I restriction enzymes (Amersham Biosciences) for 2 h at 37 °C to release terminal restriction fragments that were then subjected to