Characterisation of Leishmania telomeres reveals unusual telomeric repeats and conserved telomere-associated sequence

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ABSTRACT

Characterisation of the telomeres of Leishmania is important for understanding many aspects of the parasitic life of this primitive protozoan and for the completion of the physical map and sequencing of the genome. After sequencing more than 300 telomere-derived clones from Leishmania braziliensis and Leishmania major, a conserved 100 bp sequence was identified immediately adjacent to the telomere at the chromosome end and was named LCTAS (Leishmania conserved telomere-associated sequence). The LCTAS contains two conserved sequence boxes, and is present in all Leishmania species studied. The organisation of the LCTAS in the telomeric region differs between L.braziliensis and L.major: in L.major the LCTASs are tandemly repeated, while in L.braziliensis the LCTAS is present as a single copy per end. Two additional TASs with 1.6 kb and 274 bp repeat structures, which are apparently different to LCTAS, were isolated and mapped onto a L.braziliensis 250 kb multicycopy minichromosome and the L.major chromosome 1, respectively. An unusual feature in L.braziliensis is that the telomeric repeats are often comprised of a novel tandem repeat CCTAATCCGTGGA. A ‘slippage’ mechanism for LCTAS formation is proposed in this study as an alternative way for the synthesis and maintenance of telomeres and subtelomere regions.

INTRODUCTION

The telomere is the terminal structure at the ends of all linear eukaryotic chromosomes. Telomeric DNA typically consists of tandem arrays of short repeat sequences with the G-rich strand running 5′ to 3′ toward the end of the chromosome; telomeric repeats are conserved among distantly related eukaryotes. In most organisms that have been investigated, telomeric repeats are synthesised by telomerase, except in Drosophila species where the telomeres are capped by retrotransposon sequence repeats, synthesised by a reverse transcriptase (1,2). The subtelomeric region (sequence immediately adjacent to the telomere, called telomere-associated sequence, TAS) contains complex repeats and multiple genes. A number of TASs from different species have been studied. Saccharomyces cerevisiae telomeric associated sequences contain two types of repeat, X and Y, that are located immediately proximal to the telomeric repeats (3). In Caenorhabditis elegans, the sequences of 11 of 12 telomeric regions revealed no sequence in common (4).

Work on the trypanosomatid protozoa, which include human and domestic parasites Trypanosoma and Leishmania species, first identified the telomeric repeat sequence (CCCTAA)n in Trypanosoma brucei (5,6) and the same repeats have now been found in all vertebrate cell chromosome ends studied. In T.bruci, two interesting phenomena are associated with telomeres: antigenic variation and subtelomeric DNA modification (7,8). Two subtelomeric segments from T.bruci were cloned and analysed (9). Two candidate protein complexes which bind double-stranded telomere sequence have been identified in T.bruci (10,11). In Leishmania infantum, a subtelomeric 81 bp repeat sequence was identified on chromosomes 1 and 5 (12). Yet despite the early discovery of CCCTAAₙ telomeric repeats in T.bruci, the progress of research on the telomeres of the trypanosomatid protozoa is far behind that in other species. Little is known about the structure of the chromosome end, the subtelomeric regions and the telomerase in these primitive protozoan organisms.

We attempt here to explore the structure of the chromosome ends in Leishmania species. The protozoan Leishmania is a causative agent of Leishmaniasis, a disease which threatens 350 million people in 88 countries around the world (13). We were particularly interested in Leishmania telomeres for a variety of reasons. Firstly, the primitive taxonomy and unusual molecular biology features of Leishmania species, such as trans-splicing, RNA editing, gene amplification and chromosome size polymorphism, render them very important for basic molecular and clinical study. Secondly, characterisation of Leishmania telomeric regions is useful for the Leishmania genomic sequencing project, initiated by the World Health Organisation (14). A Leishmania major Friedlin cosmid library was previously constructed in our laboratory and made available as the reference library for global mapping and sequencing. However, chromosome ends are notably missing from the map because the methods used to

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construct the library were biased against the isolation and mapping of terminal clones. Cloning the *Leishmania* telomeres will allow completion of the physical map and the genome sequencing.

Thirdly, there are a number of interesting features with which telomeres may be associated in *Leishmania* species. There are no two species or strains from *Leishmania* which have the same karyotype pattern in PFGE analysis (15,16), indicating a high degree of chromosome plasticity and frequent recombination. Characterisation of the TAS regions may shed light upon the nature of this recombination and chromosome polymorphism. In *T. brucei*, the VSG genes which are essential for the parasite’s survival in the bloodstream of the host are predominantly located in subtelomeric regions. The gene composition of the subtelomeric region in *Leishmania* species has not been identified so far. Research on mitosis and meiosis in *C. elegans* has indicated that the telomere may play a role in chromosome attachment to the nuclear membrane and spindle during chromosome segregation (17). The cloning and characterisation of *Leishmania* telomeric regions may also provide a starting point for the study of chromosome elements mediating a similar process in *Leishmania*.

The telomerase enzyme and telomerase RNA have important functions in eukaryotic organisms, and have been studied extensively in recent years. Since the trypanosomatid protozoa diverged early in the evolution of eukaryotes, and yet they have the same telomeric repeats as vertebrates; an investigation of their functions in eukaryotic organisms, and have been studied (17). The cloning and characterisation of *Leishmania* telomeric regions may also provide a starting point for the study of chromosome elements mediating a similar process in *Leishmania*.

In this paper, we report the cloning and sequencing of a number of TAS regions, mainly from *Leishmania braziliensis* and *L. major*, but also from *Leishmania mexicana* and *Leishmania lainsoni*. A novel cloning technique was used for this project (18), which selected for telomeric sequences. This project revealed a conserved TAS present in all *Leishmania* studied.

**MATERIALS AND METHODS**

**Parasite culture and DNA preparation**

The *Leishmania* strains used in this study are: *L. major* LV39 (MRHO/SU/59/LV39), *L. braziliensis* M2903 (WHOM/BR/75/M2903), *L. lainsoni* M6426 (WHOM/BR/81/M6426), and *L. mexicana* BEL21 (MHOM/BZ/82/BEL21). The *in vitro* culture of *Leishmania* strains and the preparation of parasite DNA have previously been described (19).

**Bal31 nuclease digestion**

*Bal31* nuclease digestion was carried out as described by Wicky et al. (4).

**Cloning of telomere-associated DNA and sequencing**

Cloning of telomere-associated DNA was performed using a novel PCR-based method, Primer Tagged Amplification (PTA) (18). Sequencing was carried out using the Prism Dye terminator sequencing Kit (Perkin Elmer) and an ABI 373A automated sequencer.

**Probe preparation and hybridisation conditions**

Probes were radiolabelled by random priming (Stratagene). The specific probes for 15 TASs (tel01–15) of *L. braziliensis* were prepared as follows. The oligonucleotide primers designed to be specific for different TASs in combination with vector primer T3 were used for PCR amplification using reagents from a GeneAmp kit (Perkin Elmer). The primers are:

- tel1, 5’-ATCTCACCAGTGAGACCTCG-3’
- tel2, 5’-GCCCAAGAAGAAGTCGCT-3’
- tel3, 5’-CTGACTTTGTTGTTGTTGC-3’
- tel4, 5’-GATCCTAGACGGGCTGTA-3’
- tel5, 5’-GTGTAACCCTGCTCTTCAC-3’
- tel6, 5’-GTACGTTGGCCATTTTCAG-3’
- tel7, 5’-CATACCGTGTAGCGTGAA-3’
- tel8, 5’-ATTGGAATCGTCCACCTAAATG-3’
- tel9, 5’-CTGTTCCGGCCACCCCAC-3’
- tel10, 5’-AGGAGCAATCAAGTCTGTC-3’
- tel11, 5’-ATCAAGAAGCCGTGTCGGCCA-3’
- tel12, 5’-CTGTTACCCGTGATCGATT-3’
- tel13, 5’-TGCTTGTGCCA TGGA TCGC-3’
- tel14, 5’-TGACGCTA TGCGA TAAGCAAG-3’
- tel15, 5’-GATGCTATGGGATCCAG-3’

The PCR products were gel-purified using a Wizard PCR purification kit (Promega) and were radiolabelled for hybridisation. Other probes were gel-purified whole inserts from a clone containing the *L. major* LCTAS (*Leishmania* conserved telomere-associated sequence) dimer repeat (LCTAS probe) and a clone containing MTAS274 dimer repeat (major10 probe). All hybridisations were carried out in 0.5 M Na2HPO4, 7% SDS and 1% BSA overnight (20). The blots were hybridised at 65°C and washed in 2× SSC and 0.1% SDS at 65°C for 15–60 min.

**RESULTS**

**Cloning of Leishmania telomeres and grouping of TAS clones into unique sequence classes**

Previous studies have shown that *Leishmania* telomeric DNA has the same tandem arrays (CCCTAA)n as *T. brucei*. Based on this telomeric sequence we developed a simple and efficient method (PTA) for cloning a large number of TASs from *Leishmania* (18). We used this method to clone TASs from *L. braziliensis*, *L. major*, *L. mexicana* and *L. lainsoni*. Here we concentrate on the analysis of *L. braziliensis* and *L. major*.

A total of 262 positive clones from the four PTA-generated TAS libraries of *L. braziliensis* M2903 and 53 positive clones from the four TAS libraries of *L. major* LV39 were picked after PCR screening and sequenced. Homologous sequences were clustered into distinct groups. The 262 *L. braziliensis* clones were first divided into two groups: 151 clones containing a 100 bp conserved sequence (LCTAS) or sequence with homology to LCTAS, and 111 clones with no LCTAS sequence. Based on the unique sequence adjacent to the LCTAS, the 151 clones were further grouped into 15 classes, named tel01–15. The number of clones in each class of tel01–15 are 16, 23, 34, 13, 16, 8, 11, 2, 3, 9, 3, 5, 3, 2 and 3, respectively. Among these 15 TAS classes there are two TASs (tel010 and tel015), which contain only slight sequence homology to the LCTAS. Similarly, 53 *L. major* clones were divided into two groups: 31 clones containing the LCTAS, and 22 without. The 31 LCTAS clones were grouped into 10 unique
A probe DNA containing a single LCTAS was cut from a telo1 showing that it mapped onto the smallest 300 kb chromosome of by hybridised to the same filters shown in the lower parts of the repeat may be located only in a few (probably just one) main deleted band was observed, suggesting this 274 bp TAS chromosome ends, a series of To test whether the cloned sequences are indeed located at a filter of a major10 probe made from one of these clones was hybridised to genomic DNA followed by HindIII digestion was carried out and the filter was hybridised with probes telo2 (C), telo1 (D) and telo9 (E). The Bal31-sensitive fragments are indicated by arrows.

TAS classes, major1–10, with number of clones in each class 6, 5, 4, 1, 2, 1, 2, 3 and 5, respectively.

**Bal31-sensitive analysis of Leishmania TASs**

To test whether the cloned sequences are indeed located at chromosome ends, a series of Bal31 exonuclease sensitive analysis were performed. *Leishmania major* genomic DNA was Bal31-treated for a range of time intervals and then digested with HindIII. The gel-separated digest products were Southern blotted and hybridised with the LCTAS probe from *L. major*. Figure 1A shows that this LCTAS probe hybridised to all the bands sensitive to Bal31, indicating that the LCTASs are located at the ends of chromosomes and are probably distributed in many chromosomes. Clones of major10 contain sequences with 274 bp repeats. The major10 probe made from one of these clones was hybridised to a filter of a Bal31 digest followed by SalI digest (Fig. 1B). One main deleted band was observed, suggesting this 274 bp TAS repeat may be located only in a few (probably just one) chromosome ends. As a control, a *L. major* 18S rDNA probe was hybridised to the same filters shown in the lower parts of the panels in Figure 1A and B, revealing constant bands not digested by Bal31. A PFG blot with separated low molecular weight chromosomes of *L. major* was hybridised with the major10 probe, showing that it mapped onto the smallest 300 kb chromosome 1 (data not shown).

Similar hybridisation experiments were applied to *L. braziliensis*. A probe DNA containing a single LCTAS was cut from a telo1 class clone, and was hybridised to a Bal31 digest gel. Multiple digested bands and multiple undigested bands were revealed (data not shown). Because the multiple deleted bands were embedded in multiple undeleted bands, which were hardly visible, we decided to perform hybridisation using unique sequence probes for each TAS class. Different restriction enzymes were used to digest Bal31-treated *L. braziliensis* genomic DNA, and the blotted gels were hybridised with the 15 individual probes. We found that most of these probes detected at least one sensitive band deleted by Bal31 digestion. Some of these results are shown in Figure 1. The telo2 probe detects one deleted band and one undeleted band (Fig. 1C); the telo1 probe detects two deleted bands and two undeleted bands (Fig. 1D); the telo9 probe detects two deleted bands (Fig. 1E). The telo10 probe was mapped onto the 250 kb, highly amplified minichromosome by a PFG hybridisation, and detected multiple deleted bands in Bal31-sensitive analysis (data not shown).

**Structural organization of Leishmania TASs**

Many LCTAS clones from *L. major* were found to contain dimers or trimers of the 100 bp LCTAS, while all LCTAS clones from *L. braziliensis* contain a single copy LCTAS. To test whether the LCTAS is organised as a tandem repeat in the *L. major* genome, we partially digested genomic DNA with the restriction enzyme *AvrII* which cuts the LCTAS internally. The blotted gel was hybridised with the LCTAS probe. Figure 2A shows a 100 bp ladder hybridisation signal, indicating that the LCTAS is indeed organised as a tandem repeat in *L. major*.

A similar experiment was done using *L. braziliensis* genomic DNA, partially digested with the restriction enzyme *BspOI*, which cuts the beginning of M2903 LCTAS. Hybridisation with the LCTAS probe suggested that *L. braziliensis* LCTAS is not
Figure 3. Sequence alignments of TASs. (A) An alignment of 15 different TASs of *L. braziliensis* M2903. (B) An alignment of 10 different TASs of *L. major* LV39. (C) An alignment of TASs from four *Leishmania* species. See text for the name details. (D) An alignment of four TASs of class telo5 of *L. braziliensis* M2903. Telomeric repeats are in italic and underlined. Two conserved sequence boxes CSB1 (GTACAGT) and CSB2 (GGAGAGGGTGT) are underlined. N represents any nucleotide; n represents any number; numbers 1, 2 and 27 in sequences indicate the repeat numbers; m indicates numbers ranging from 1 to 51.

organised as a tandem array because no ladder signal was observed (data not shown).

The sequence from major10 clones was also tested for repeat structure. A partial digest of genomic DNA with *Kpn*I which cuts the major10 sequence internally and hybridisation with the major10 probe showed that this sequence is organised into a 274 bp tandem repeat structure (Fig. 2B) (named MTAS274 repeat, major telomere-associated sequence 274 bp repeat).

The sequence from telo10 clones of *L. braziliensis*, which maps onto the minichromosome, was also tested for repeat structure. Two partial digestions of genomic DNA with *Sph*I and *Kpn*I, which cut the telo10 sequence internally, were hybridised with the telo10 probe. A 1.6 kb ladder signal was observed in both gels (Fig. 2C and D), suggesting the telo10 sequence is organised into a 1.6 kb repeat structure (named MINI-TAS, minichromosome telomere-associated sequence).

Sequence analysis of *Leishmania* TASs

From sequence comparison of LCTASs from different *Leishmania* species we can define two conserved sequence boxes: GTA-CAGT (CSB1) and GGAGAGGGTGT (CSB2). The space between the two boxes varies from 1 to 51 bp. Downstream of the two conserved sequence boxes, one or two CCCTAA repeats link the LCTAS with unique sequences. Figure 3A shows the alignment of 15 TAS classes from *L. braziliensis*. We can see that the 100 bp TASs are highly conserved apart from telo10 and telo15. However, even in telo10 (from the minichromosome) and telo15 of unknown location, one still can see weak homology in the two conserved sequence box regions.

Figure 3B shows an alignment of 10 TASs of *L. major*. Unlike *L. braziliensis*, the LCTAS sequences are tandemly repeated, and the two consensus sequences are spaced by 43 or 51 bp. In *L. major* the 274 bp tandemly repeated MTAS274, which maps onto chromosome 1, was also found to have weak homology to LCTAS over the two conserved boxes.

Figure 3C shows a comparison of several TASs, including the LCTAS consensus sequence of *L. braziliensis* (M2903con), the LCTAS consensus sequence of *L. major* (majorcon), the TAS major8 of *L. major*, two *L. lainsoni* TASs, one *L. mexicana* and one *Leishmania donovani* TAS. The *L. lainsoni* and *L. mexicana* TASs are part of ongoing work, while the *L. donovani* TAS is from previous work by Ellis et al. (21). These four sequences were not tested for *Bal*31 sensitivity, but were suggested to be located at chromosome ends. From this comparison we can define a consensus sequence shown in the ‘consen’ line of Figure 3C. The two boxes are highly conserved between different species; the absence of CSB1 in the *L. donovani* TAS may be due to the cloning process. Unusually in *L. donovani* the sequence following the LCTAS is 27 repeats of GGGTTA instead of the usual one to three CCCTAA repeats. It is not clear if this represents the exact nature of the chromosome end or if it is a cloning artifact. The LCTASs from different species apparently have a common origin. The similarity between the LCTASs of different species is
consistent with the taxonomic relationships of the species: *L.brazilensis* and *L.lainsoni* are closed related *brazilensis* complex species, *L.mexicana* is close to *L.donovani*, and both *L.mexicana* and *L.donovani* are related to *L.major* (22 and Fu et al., unpublished data).

An unusual feature of *L.brazilensis* telomeric DNA is that some chromosome ends contain a number of tandem arrays of 14 bp CCCCTAACCCGTGGA repeats (Fig. 3A). This 14 bp repeat may solely constitute some native chromosome ends, but other chromosome ends contain a mixture of two types of repeats, organized in order of CCCCTAA repeats—CCCTAACCCGTGGA repeats—LCTAS. The junction sequence between the telomeric repeat (including CCCCTAA and CCCTAACCCGTGGAA) and the LCTAS is always CCCTAACCCGTGGA except in telo10 and telo15 (Fig. 3A), indicating a relationship between the two types of repeat.

When we compared sequences from the same classes, we found that sequences in each class were not always identical. Figure 3D shows an example of four telo5 sequences, where the sequence differences between them are apparent. It seems unlikely that this is due to PCR errors during amplification, since the frequency of mutation among some sequences is so high and clearly not random. It is most likely that their differences reflect the real nature of the chromosomes, implying that some different chromosomes share the same end sequence in which the mutation has already occurred. This suggestion is supported by the observation that some TASs detected more than one deleted band in *Bal31* sensitive analysis experiments (Fig. 1D and E).

**The novel telomeric organisation of the *L.brazilensis* M2903 minichromosome**

Clone telo10 from *L.brazilensis*, which was found to map onto the highly amplified minichromosome, was analysed further. A 5 kb subtelomeric region of the minichromosome was cloned and sequenced. Analysis of these sequences revealed a number of features. The 5 kb subtelomeric sequence of the minichromosome shows the sequence to be a 1.6 kb dimer repeat of MINI-TAS which is also shown in (B). The ends of some other chromosomes share the same sequence in which the mutation has already occurred. This suggestion is supported by the observation that some TASs detected more than one deleted band in *Bal31* sensitive analysis experiments (Fig. 1D and E).

**A general model of *Leishmania* chromosome ends**

Based on above sequence analysis we can define a general *Leishmania* chromosome end model which is shown in Figure 4B–E. In *L.brazilensis* M2903, some chromosomes end in CCCCTAA tandem repeats, followed by 14 bp CCCCTAACCCTAACCCGTGGA repeats as we move inwards, then the 100 bp LCTAS and finally unique sequence for the individual chromosome end. The names of different TAS repeats are written above the bars they represented. In (B) and (C) the heavy lines represent the regions where sequences share some homology. The filled arrowhead and arrowhead followed by an open box represent telomeric repeats CCCCTAA and CCCCTAACCCGTGGA, respectively. The LCTAS and MTAS274 repeats in (D) and (E) do not represent precise numbers. The diagram is not to scale.
The LCTAS in *L. major* is tandemly repeated. The sequence comparison shows that the LCTAS repeats in *L. major* are not identical at different chromosome ends; some deletions and mutations occur between them (Fig. 3B). However, analysis of a number of clones which contain several LCTAS repeats revealed that repeats in individual clones are always identical to each other, in other words, different LCTAS repeats are never mixed up at one chromosome end. Two clones (5A24 and 5A56), for example, contain two and four LCTAS repeats, respectively, which are almost identical within the clone, but different between the clones (Fig. 5A). This suggests that each type of repeat has a single recent origin. These observations lead us to put forward a ‘slippage’ mechanism for the formation of LCTAS repeats. The most plausible explanation for an increase in repeat number is that the end LCTAS repeat slips back one unit and initiates polymerisation to synthesise one more repeat. A detailed description of this ‘slippage’ is illustrated in Figure 5B. The CCCTAA telomeric repeats which link two LCTASs range in number from 2 to 11 repeats, these may be created by telomerase adding a few CCCTAA repeats before the LCTAS slippage occurs; or by choosing different priming sites during CCCTAA and GGGA TT repeats annealing after slippage. Secondary structure analysis of LCTAS revealed that several hairpin stems could form which may promote the LCTAS slippage, supporting the above hypothesis. Further experiments, such as transfection of LCTAS and analysis of its *in vivo* behaviour, may provide more insight into this mechanism.

**DISCUSSION**

The characterisation of the 100 bp LCTAS in *L. braziliensis*, *L. major*, *L. mexicana*, *L. donovani* and *L. lainsoni* indicates that it is a conserved DNA element among different species. The two highly conserved sequence boxes, CSB1 and CSB2, were found to be present on almost all chromosome ends studied. In *L. major* the LCTASs are organised in tandem repeats and there are several copies of CCCTAA sequence present between the LCTAS repeats. This structure is strikingly similar to that of the chromosomal extremities of *Saccharomyces cerevisiae* and *Plasmodium berghei*, in which telomeric sequences are intercalated between the 6–7 kb Y′ repeats and 2.3 kb repeats, respectively (3,25,26). Thus, the structure of subtelomeric repeats intercalated by telomeric sequences also seems to be conserved between different organisms.

Based on sequence analysis we put forward a ‘slippage’ hypothesis to explain LCTAS repeat formation. At present, there are two mechanisms for the maintenance and protection of chromosome ends: the adding of telomere repeats by telomerase in most eukaryotes and capping retrotransposon by reverse transcriptase in *Drosophila*. If the ‘slippage’ mechanism is confirmed by further experiments, it will provide an alternative way for the synthesis and maintenance of telomeres and subtelomere regions. Furthermore, this mechanism may be not limited to *Leishmania* species.

In *L. braziliensis*, in addition to the conventional 6 bp CCCTAA telomeric repeat, there is another telomeric repeat, CCCTAAC-CCTGCGA, present on some chromosome ends. Our previous
work has shown that in L. Lainsoni some chromosomes contain telomere repeats other than the usual repeat, since a telomeric repeat probe failed to detect these chromosomes by hybridisation (18). The unusual telomeric repeats may be replicated by telomerase using a different telomerase RNA template which either coexists in the genome with the conventional one or is an extended version of it, producing a longer template. If this is true, the telomerase RNA gene in L. braziliensis could contain a stretch of CCCTAACCCGTTGGA.

Leishmania braziliensis minichromosome ends show a different sequence composition and a novel organisation. Previous study demonstrated that this chromosome is a LD1-related highly amplified minichromosome, which is organised as a big inverted structure (27). When the minichromosome DNA was digested with different restriction enzymes and hybridised with a telomeric probe, the signal was always one band. This indicates that, like recently discovered telomere length-control mechanisms in human and yeast, the length of the telomere in Leishmania is also highly controlled. This implies that the telomerase (if it is present) always synthesises the same length of telomere DNA at both ends of the minichromosome; a length control mechanism which must be able to recognise both types of telomeric repeat (of 6 and 14 bp).

The high degree of conservation of the LCTAS among different species suggests that it could play an important role in the biology of these parasites. The two conserved LCTAS boxes may act as telomere adding signals, since they are present on almost every chromosome. However, studies on C. elegans telomere DNA have shown that the telomere DNA sequence alone is sufficient for telomere replication (4), implying that the LCTAS may have an alternative function. It is likely that the LCTAS repeat in L. major is the main source of chromosome size polymorphism due to differing LCTAS number, since subtelomeric sequences are commonly believed to be involved in recombination and act as buffer zones between the chromosome end and the most distal genes.

In this work, we found that the LCTAS was absent on the multicopy minichromosome of L. braziliensis, replaced by the MINI-TAS repeat. This leads us to suspect that the MINI-TAS and LCTAS could have a bearing on chromosome copy number. The TAS repeat may act as a centromere-related element, important for chromosome segregation. Several recent advances favour the idea that telomeres are involved in mitotic and meiotic chromosome segregation (17,28). We are currently involved in artificial chromosome construction and transfection experiments using the conserved TAS repeat of L. major as a telomere, in an effort to gain more insight into the role of the TAS repeat in Leishmania. The LCTAS is probably a recognition site for protein binding, and identification of LCTAS binding proteins should reveal more about the function of the chromosome end.

In this paper, we report the first step in the molecular analysis of Leishmania telomeres. To our knowledge, we have identified the first conserved non-coding DNA element in Leishmania genomic DNA, with the exception of telomeric repeats. The TAS will be useful as a sequence marker for characterisation of species and their taxonomic relationship within Leishmania. In addition, we have provided a helpful basic resource towards completion of the Leishmania genomic DNA mapping and sequencing project.

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