The *Leishmania amazonensis* TRF (TTAGGG repeat-binding factor) homologue binds and co-localizes with telomeres

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**Abstract**

**Background:** Telomeres are specialized structures at the end of chromosomes essential for maintaining genome stability and cell viability. The importance of telomeric proteins for telomere maintenance has increased our interest in the identification of homologues within the genus *Leishmania*. The mammalian TRF1 and TRF2 proteins, for example, bind double-stranded telomeres via a Myb-like DNA-binding domain and are involved with telomere length regulation and chromosome end protection. In addition, TRF2 can modulate the activity of several enzymes and influence the conformation of telomeric DNA. In this work, we identified and characterized a *Leishmania* protein (LaTRF) homologous to both mammalian TRF1 and TRF2.

**Results:** LaTRF was cloned using a PCR-based strategy. ClustalW and bl2seq sequence analysis showed that LaTRF shared sequence identity with the *Trypanosoma brucei* TRF (TbTRF) protein and had the same degree of sequence similarities with the dimerization (TRFH) and the canonical DNA-binding Myb-like domains of both mammalian TRFs. LaTRF was predicted to be an 82.5 kDa protein, indicating that it is double the size of the trypanosome TRF homologues. Western blot and indirect immunofluorescence combined with fluorescence in situ hybridization showed that LaTRF, similarly to hTRF2, is a nuclear protein that also associates with parasite telomeres. Native and full length LaTRF and a mutant bearing the putative Myb-like domain expressed in bacteria bound double-stranded telomeric DNA in vitro. Chromatin immunoprecipitation showed that LaTRF interacted specifically with telomeres in vivo.

**Conclusion:** The nuclear localization of LaTRF, its association and co-localization with parasite telomeres and its high identity with TbTRF protein, support the hypothesis that LaTRF is a *Leishmania* telomeric protein.

**Background**

More than 20 *Leishmania* species are pathogenic to humans and cause leishmaniasis of differing severity. *Leishmania amazonensis* (Trypanosomatidae), the parasite studied in this work, is common in Brazil and causes a wide spectrum of clinical leishmaniasis [1]. The parasite can cause opportunistic infections in HIV/AIDS patients and co-infections have been reported in 34 countries. There are no adequate methods for controlling leishmaniasis and current available treatments are inefficient [2,3]. Consequently, most of the ongoing research for new drugs to combat the disease is based on post-genomic approaches [4].

Telomeres are specialized structures at the end of chromosomes and consist of stretches of repetitive DNA (5'–TTAGGG–3' in vertebrates and trypanosomatids) and associated proteins [5]. Telomeres are essential for maintaining genome stability and cell viability, with dysfunctional telomeres triggering a classic DNA-damage response that enables double-strand breaks and cell cycle arrest [6].

There are three classes of telomeric proteins, viz., proteins that bind specifically to single-stranded G-rich DNA, proteins that bind to double-stranded DNA and proteins that interact with telomeric factors. Other non-telomeric proteins, such as the DNA repair proteins...
Results and Discussion

Characterization of the putative L. amazonensis TRF gene homologue

Using data mining via the OmniBLAST server we searched the whole L. major genome database http://www.ebi.ac.uk/parasites/leish.html for a putative sequence that shared similarities with the vertebrate TRF1 and TRF2 proteins. For this search, we used the most conserved part of both human proteins, the C-terminal fragment containing the Myb-like DNA binding domain. The search returned a single sequence (GenBank acc. no. XP_001682531.1) that encoded a hypothetical protein (GenBank acc. no. Q4QDR7, GeneDB_Lmajor LmjF18.1250), the C-terminus of which shared ~30% identity and 50-55% similarity with the vertebrate TRF Myb-like domain, according to the blast2 sequence analysis (Table 1).

Based on the L. major sequence, primers were designed for PCR amplification of the entire homologous sequence from L. amazonensis with genomic DNA as the template. PCR products of 2,931 bp were cloned into the vector pCR2.1 and both insert strands were sequenced (data not shown). The deduced polypeptide sequence of 796 amino acid residues contained a putative C-terminal Myb-like DNA binding domain between residues 684-733, according to psi-blast (Fig 1 - top). The LaTRF gene (GenBank acc. no. EF559263) shared high sequence identity and similarity to the putative L. major TRF, and to hypothetical L. infantum and L. braziliensis TRFs (Table 1). The sequence conservation between LaTRF and the trypanosome TbTRF and the putative TcTRF homologues decreased to 35-45% identity (Table 1), consistent with the known evolutionary relationships among these organisms. The Leishmania TRF homologues encode the largest TRF protein (~82.5 kDa) described so far. The fact that the Leishmania proteins showed much greater homology with each other than with other protozoan proteins and that they are the largest TRF described so far resembles the situation for Leishmania telomerase protein [25].

In addition, like TbTRF, LaTRF shared sequence similarities with the canonical Myb-like domain and with the TRFH dimerization domain of human TRF1 and TRF2 (Fig 1-bottom and Table 1), but no sequence similarities were found with any other telobox protein (data not shown). Together, these results indicate that although LaTRF shares high sequence similarity with TbTRF, probably because the two species are phylogenetically related [26], further studies are required to confer any functions to the Leishmania TRF homologue identified here.

LaTRF is a nuclear protein that co-localizes with L. amazonensis telomeres

In exponentially growing L. amazonensis promastigotes, LaTRF was detected only in nuclear protein extracts. A single ~82.5 kDa protein band was detected using anti-LaTRF serum (Fig 2 - top panel: lane 1). No protein was detected in cytoplasmic and total protein extracts (Fig 2 - top panel: lanes 2 and 3), indicating that LaTRF is a nuclear protein with very low intracellular abundance. As
a control, Western blots were revealed with anti-LaRPA-1 serum, which recognizes a ~51.2 kDa telomeric protein band [23] (Fig 2 - bottom panel: lane 1) and also its phosphorylated forms (Fig 2 - bottom panel: lane 2; da Silveira & Cano, unpublished data).

We also developed an immunofluorescence assay combined with FISH, using anti-LaTRF serum and a PNA-telomere probe specific for TTAGGG repeats. As shown in Fig 3 (panels p1-4, merged images a and b), LaTRF is a nuclear protein that partially co-localizes with parasites telomeres, since some of the LaTRF signal coincided with telomeric foci and some did not (Fig 3, panels p1-4). In most cells, LaTRF appears as a diffuse signal spread all over the nucleoplasm and only in some cases it forms large punctuated foci, which seems to co-localize with the telomeric DNA (yellow dots in Fig 3, panels p2 and p4). Similarly, in humans, the hTRF2 protein also appears in the form of punctuate foci that does not completely associate with telomeres [18], which is in agreement with other cellular functions played by this protein [27,28]. In contrast, the T. brucei TRF protein (TbTRF) appears to co-localize with most telomeres at all stages of the cell cycle.

Table 1: Pairwise analysis of amino acid sequence alignments from TRF homologues based on bl2seq sequences (protein-protein BLAST)

|                | LaTRF (full length) | LaTRFTRFHdomain | LaTRFMybdomain |
|----------------|---------------------|-----------------|----------------|
|                | %Identity           | %Similarity     | %Identity      | %Similarity     | %Identity      | %Similarity     |
| LmTRF          | 99                  | 99              | 100            | 100             | 100            | 100             |
| LiTRF          | 88                  | 91              | 85             | 89              | 98             | 100             |
| LbTRF          | 65                  | 71              | 60             | 70              | 96             | 100             |
| TcTRF          | 45                  | 59              | 38             | 54              | 63             | 77              |
| TbTRF          | 35                  | 53              | 39             | 59              | 54             | 66              |
| hTRF1          | Not significant     | Not significant | 16             | 25              | 31             | 54              |
| hTRF2          | Not significant     | Not significant | 15             | 30              | 29             | 55              |

La, L. amazonensis (GenBank acc. no. EF559263); Lm, L. major (TrEMBL acc. no. Q4QDR7); Li, L. infantum (GenBank acc. no. XP_001464939.1); Lb, L. brasiliensis (GenBank acc. no. XP_001364056.1); Tc, Trypanosoma cruzi (GenBank acc. no. XP_819954.1); Tb, Trypanosoma brucei (GenBank acc. no. AY910010); h, human (hTRF1 GenBank Acc. no. P54274.2; hTRF2 GenBank acc. no. Q15554).

Figure 1 LaTRF is a homologue of mammalian and T. brucei telomeric TRFs (top) Position of the TRFH and Myb domains in LaTRF, according to rpsblast and bl2seq sequence analysis with T. brucei TRF. (bottom) ClustalW multiple alignment of the Myb-like DNA binding domains of human (hTRF2 and hTRF1), L. amazonensis (LaTRF), T. brucei (TbTRF) and T. cruzi (TcTRF) TRFs.
cycle in both bloodstream and procyclic forms [24]. Whether LaTRF also has other cellular roles or if its association with telomeres occurs in a cell cycle dependent manner is not clear at this stage.

LaTRF interacts in vitro and in vivo with L. amazonensis telomeres using a Myb-like DNA binding domain

EMSA assays were done with renatured protein extracts containing full length LaTRF, the Myb-like DNA binding domain (LaTRF\textsuperscript{Myb}) (Figs 4 and 5, see additional file 1) and with L. amazonensis nuclear extracts (Fig 6), to investigate whether LaTRF, like its vertebrate and trypanosome counterparts [18,24], was able to bind double-stranded telomeric DNA \textit{in vitro}.

The full-length recombinant protein and its deletion mutant were expressed in very low amounts and in non-soluble form in the \textit{E. coli} system (data not shown) making their purification by conventional chromatography very difficult. Therefore, protein expression was checked by Western blot using anti-LaTRF serum and anti-His tag monoclonal antibody (data not shown).

As shown in Fig 4, recombinant full length LaTRF and the mutant bearing the C-terminal Myb domain bind \textit{in vitro} double-stranded telomeric DNA. Electrophoretic mobility shift assays (EMSA) were done using radiolabeled double-stranded telomeric DNA (LaTEL) as probe. Protein-DNA complexes were separated in a 4% PAGE in 1X TBE. EMSA was done with \textit{E. coli} BL21 protein extract (lane 2), recombinant full length LaTRF (lanes 3-6) and a mutant bearing the C-terminal Myb domain (lanes 7-9). A supershift assay was done with anti-LaTRF serum (lane 6). Assays were also done in the presence of 20 fold excess of non-labeled LaTEL as specific competitor (lanes 4 and 8) or 100 fold excess of double-stranded non-specific poly [dI-dC] [dI-dC] DNA (lanes 5 and 9). In lane 1, no protein was added to the binding reaction. The original gel image and its content are shown as additional file 2: Figure S1.
excess unlabeled LaTEL (Fig 5, lane 3) and no competition was detected in the presence of non-specific DNA (Fig 5, lane 4). The results presented above suggest that recombinant LaTRF binds LaTEL potentially via the putative Myb-like DNA binding domain indicating a role for the C-terminal region of LaTRF in mediating sequence-specific binding to telomeric DNA.

Nuclear extracts were obtained from log phase *L. amazonensis* promastigotes in order to check if native LaTRF was also able to bind double-stranded telomeric DNA (LaTEL) as probe. Protein-DNA complexes were separated in a 4% PAGE in 1X TBE. EMSA was done with recombinant full length LaTRF and anti-LaTRF serum in the absence (lane 2) and in the presence of 20 fold excess of non-labeled LaTEL as specific competitor (lane 3) or 100 fold excess of double-stranded non-specific DNA (poly [dI-dC] [dI-dC]) as non specific competitor (lane 4). In lane 1 reaction was done in the presence of LaTEL only.

Figure 5 Supershift and competition assays confirm that recombinant full length LaTRF bind *in vitro* double-stranded telomeric DNA. Electrophoretic mobility shift assays (EMSA) were done using radiolabeled double-stranded telomeric DNA (LaTEL) as probe. Protein-DNA complexes were separated in a 4% PAGE in 1X TBE. In lanes 2-6, EMSA was done with nuclear extracts obtained from *L. amazonensis* promastigotes. In lane 2, the reaction was done in the absence of competitors. In lanes 3 and 4, binding reactions were done respectively, in presence of 100 fold excess of double-stranded non-specific DNA (poly [dI-dC] [dI-dC]) and 20 fold excess of non-labeled LaTEL. In lane 5, a supershift assay was done with anti-LaTRF serum and in the presence of 20 fold excess of non-labeled LaTEL and in lane 6, the supershift assay shown in lane 5 was done in the absence of competitors.

Chromatin immunoprecipitation experiments also suggested that LaTRF is a telomeric protein. The anti-LaTRF serum immunoprecipitated *L. amazonensis* telomeric DNA (Tel1) *in vivo* (Fig 7 - left) but did not immunoprecipitate the GT-rich kinetoplast DNA (kDNA) (Fig 7 - right). The kDNA control represented by the UMS (universal mini-circle sequence) albeit GT-rich, is very representative of the general base composition of *Leishmania* genomic DNA. In addition, it is a good control, since we were able to show that it was co-immunoprecipitated by two other *Leishmania* telomeric protein [17,23]. In a previous study, we described LaTBP1, a protein that specifically binds telomeric and GT-rich DNA in *Leishmania.*
LaTBP1 has a centrally positioned Myb-like DNA binding domain and is most likely a non-telobox protein that is apparently related to the multifunctional yeast RAP1 telomeric protein and TFIIIB B’ transcription factor [17]. Together with the putative LaTRF described here, these are the only descriptions of proteins bearing a Myb-like DNA binding domain that interact with double-stranded telomeric DNA in Leishmania.

As mentioned here and elsewhere [26], the huge evolutionary distance between this protozoan and higher eukaryotes presents a barrier when searching for protein homologues in the genomes of these parasites. For example, no TRF1 homologues were found in trypanosomatid genomes but the expression of hTRF1 in procyclic forms of T. brucei caused telomere shortening and cell cycle arrest, probably by displacing an unknown endogenous telomeric factor [29]. RNAi knockdown of TbTRF arrested bloodstream cells in G2/M and most of the procyclic forms were arrested in the S phase, and caused shortening of G-rich single-stranded telomeric DNA. These findings suggest that TbTRF is probably the unknown endogenous telomeric factor, which resembles the function of mammalian TRF2 at parasite telomeres [24]. The functions of LaTRF at Leishmania telomeres remain to be determined.

Conclusions
In this report we describe the characterization of the Leishmania TRF homologue and show that it is the largest TRF protein homologue described so far. This protein contains a canonical C-terminal Myb-like DNA binding domain as well as a putative and less conserved TRFH dimerization domain [30]. In addition, LaTRF is expressed exclusively in the nucleus and like its vertebrate and trypanosome counterparts, binds to parasite telomeres in vitro and in vivo. It can also co-localize with parasite telomeres, despite being spread all over the nucleoplasm in most cells, suggesting that LaTRF may play additional cellular roles beyond its possible telomeric function.

Methods
Parasite cultures
L. amazonensis promastigotes (MHOM/BR/73/M2269) were grown in M199 medium (Cultilab) supplemented with 10% fetal calf serum (Cultilab), 25 mM HEPES and 1 × antibiotic/antimycotic solution (Cultilab) at 28°C.

Isolation of L. amazonensis genomic DNA and cloning of the LaTRF gene
Total genomic DNA of L. amazonensis was prepared as previously described [31]. LaTRF was cloned using a PCR-based strategy. Primers were designed based on the putative sequence LM16.2.Contig67 from L. major (GeneDB_Lmajor LmjF18.1250) for amplification of the complete LaTRF open reading frame (ORF) (See additional file 2: Table S1). The PCR product spanning the entire L. amazonensis TRF ORF (2,391 bp) was obtained by using the primers F1 and R1 and 1U of Platinum Taq (Invitrogen) followed by cloning into the pCR 2.1 cloning vector (Invitrogen). The PCR product was sequenced using specific primers and primers from the vector (See additional file 2: Table S1). The primers F1 and R1 contained restriction sites for NdeI and XhoI (See additional file 2: Table S1) to allow further cloning of the gene in-frame with a N-terminal 6x His-tag into plasmid pET-28a+ (Novagen).

Amino acid sequence alignments were done with blastp, blastn, and ClustalW using default parameters. The sequences used for these analyses were: hTRF2 (GenBank acc. no. Q15554), hTRF1 (GenBank acc. no. P54274.2), TbTRF (GenBank acc. no. AY910010), putative LmTRF (TrEMBL acc. no. Q4QDR7, GeneDB_Lmajor LmjF18.1250), TcTRF (GenBank acc. no. XP_819954.1), LiTRF (GenBank acc. no. XP_001464939.1) and LbTRF (GenBank acc. no. XP_001564056.1). The L. amazonensis LaTRF gene sequence was submitted to GenBank and is available under the accession number EF559263.

Construction of an LaTRF deletion mutant (LaTRFMyb)
To verify the existence of a Myb-like DNA-binding domain at the C-terminus of the protein, a deletion mutant was constructed. The primers F3 and R1 (See additional file 2: Table S1) were used to amplify the deletion mutant LaTRFMyb from genomic DNA, which contained the putative C-terminal Myb-like DNA binding domain. This mutant has approximately 665 bp that span nt 1726-2391. As with full length LaTRF, the LaTRFMyb mutant was cloned into the pCR 2.1 cloning vector.
Different protein extracts obtained from 10<sup>7</sup> parasites were prepared using RIPA buffer (150 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100 and 0.1% SDS) in the presence of 10 U of DNase I and 1X protease inhibitor cocktail (Calbiochem) and incubated for 15 min at 4°C. Cell lysates were homogenized by vortexing at maximum speed (5 bursts of 10 s each). Extracts were cleared by centrifugation at 9,300 ×g for 8 min at 4°C, to separate the total protein (supernatant) from the cellular debris (pellet). Experiments were conducted in preliminary experiments. L. amazonensis promastigotes, were standarized in pre

### Expression of LaTRF and the deletion mutant LaTRF<sub>Myb</sub> proteins in E. coli

Full length LaTRF and the deletion mutant LaTRF<sub>Myb</sub>, cloned into a pET 28a+ vector, were transformed in E. coli strain BL21 DE3 RP codon plus cells for expression in the presence of 1 mM IPTG. Both proteins were expressed in low amounts and in non-soluble form, preventing them from being purified by affinity chromatography based on the 6x His-tag. To overcome this problem, the non-soluble bacterial pellets containing both proteins were solubilized in 7 M urea, sonicated in the presence of 10 U of DNase I (Sigma) and renatured by dialysis in 50 mM glycine, pH 8.0. The presence of each protein in the extracts was checked by electrophoresis in 10% SDS-PAGE followed by Western blot probed with anti-LaTRF serum and with an anti-His tag monoclonal antibody (Novagen).

### Preparation of L. amazonensis total and nuclear extracts

Promastigotes in mid-exponential growth were used to obtain both extracts. Nuclear and cytoplasmic extracts were prepared with a Nuclear Extract Kit (Active Motif) adapted for L. amazonensis promastigotes in the presence of phosphatase and protease inhibitors.

Total protein extracts were obtained using RIPA buffer (150 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100 and 0.1% SDS) in the presence of 10 U of DNase I and 1X protease inhibitor cocktail (Calbiochem) and incubated for 15 min at 4°C. Cell lysates were homogenized by vortexing at maximum speed (5 bursts of 10 s each). Extracts were cleared by centrifugation at 9,300 ×g for 8 min at 4°C, to separate the total protein (supernatant) from the cellular debris (pellet).

Both extracts were stored at -80°C and their protein concentrations were measured by the Bradford dye-binding assay, using bovine serum albumin as standard.

### Western blot analysis

Different protein extracts obtained from 10<sup>7</sup> parasites were separated by SDS-PAGE on 10% polyacrylamide gels and transferred to nitrocellulose membranes (BIO-RAD) in Tris-glycine-methanol at 16°C. The membranes were probed with rabbit anti-TRF2 serum raised against the synthetic peptide Nt-APAVTTRKRPRSSDSP-Ct (Sigma). The extracts were also probed with anti-LaRPA-1 serum as a control [23,32]. In both cases, immunoreactive bands were revealed by using an Amplified Alkaline Phosphatase Immun-Blot Assay Kit, according to the manufacturer’s instructions (BIO-RAD).

### EMSA (electrophoretic mobility shift assay)

All of the conditions for binding reactions and EMSA, including binding temperature, protein concentrations in the extracts and the double-stranded DNA probe (LaTEL), were standardized in preliminary experiments. LaTEL was constructed by using the γ [32P]ATP 5’-end-labeled oligonucleotides ssTel78G and ssTel78C, as described by Lira <i>et al</i>. [17]. Assays were done by mixing 10 μg of renatured bacterial extracts containing full length LaTRF or LaTRF<sub>Myb</sub> with approximately 2 pmol of labeled probe (LaTEL) in 30 μl of EMSA buffer (20 mM HEPES, 2.5 mM MgCl2, 0.1 mM EDTA, 0.1 M KCl, 10% glycerol, 0.5 mM DTT, pH 8.0) containing 10 ng of poly [dl-dC] [dl-dC] and 10 ng of poly [da-dT] [da-dT]. Total protein extracts of non-transformed E. coli were used as controls. The reactions were incubated for 30 min at room temperature and loaded onto a non-denaturing 4% polyacrylamide gel (acylamide:his-acylamide, 19:1, w/w) in 1X TBE. After electrophoresis, the gels were exposed to X-ray film.

Binding reactions were also done with crude nuclear extracts obtained from 10<sup>6</sup> parasites (~2.3 μg of total proteins) and γ [32P]ATP labeled LaTEL (2 pmol) in EMSA buffer containing a mixture of 10 ng of poly [dl-dC] [dl-dC] and 10 ng of poly [da-dT] [da-dT].

Competition assays to test the binding specificity of proteins in both recombinant and nuclear extracts, were done using 20 fold excess of unlabeled LaTEL (in relation to the labeled probe) as the specific competitor and a 100 fold excess (in relation to the labeled probe) of unlabeled double-stranded DNA poly [dl-dC] [dl-dC] as the non-specific competitor.
Supershift assays were done using full-length recombinant LaTRF (10 μg) or native nuclear extracts from 10^8 parasites in the presence of ~30 μg of anti-LaTRF serum in EMSA buffer containing labeled LaTEL as probe and both poly [dI-dC] [dI-dC] and poly [dA-dT] [dA-dT] as above described. These assays were also performed in the presence of 20 fold excess of non-labeled LaTEL and 100 fold excess of poly [dI-dC] [dI-dC] as described above.

**Chromatin immunoprecipitation**

Formaldehyde cross-linked chromatin was obtained from promastigote forms of *L. amazonensis* parasites (0.8 × 10^8 cells/experiment) as described by Lira et al. [17]. Chromatin was immunoprecipitated with anti-LaTRF serum and DNA was extracted after cross-link reversal. DNA samples were slot-blotted and hybridized with Tel1 and kDNA (5’-TTCCGGTCGG-GCGGTGAAACTGCGGTTGGTGTAAAT-3’), according to Lira et al. [17].

**Additional material**

**Additional file 1** S1. Original and unmanipulated gel image shown in figure 4. EMSA done with radiolabeled double-stranded telomeric DNA (LaTEL) as probe. Protein-DNA complexes were separated in a 4% PAGE in 1X TBE. In lane 1, no protein was added to the binding reaction. In lane 2, EMSA was done with E. coli BL21 protein extract. In lane 3, EMSA was done with recombinant full length LaTRF. In lane 4, EMSA was done with recombinant full length LaTRF in the presence of 100 fold excess of non-labeled LaTEL as specific competitor. In lane 5, no protein was added to the binding reaction (as in lane 1). In lane 6, EMSA was done with recombinant full length LaTRF in the presence of 10 fold excess of double-stranded non-specific poly [dI-dC] [dI-dC] DNA. In lane 7, EMSA was done with recombinant full length LaTRF in the presence of anti-LaTRF serum (supershift assay). Please check the supershifted complex at the top of the lane. In lane 8, EMSA was done with the mutant recombinant protein bearing the C-terminal Myb domain. In lane 9, EMSA was done with the mutant recombinant protein bearing the C-terminal Myb domain in the presence of 20 fold excess of non-labeled LaTEL. In lane 10, the same experiment shown in lane 9. In lane 11, EMSA was done with the mutant recombinant protein bearing the C-terminal Myb domain in the presence of 100 fold excess of double-stranded non-specific poly [dI-dC] [dI-dC] DNA. In lane 12, the same supershift assay shown in lane 7.

**Additional file 2** Table S1 Primers used for PCR amplification and sequencing of the putative L. amazonensis TRF gene and the deletion mutant TRFMyb. Table containing a list of the primers used for PCR and sequencing assays

**Authors’ contributions**

MSS performed molecular cloning techniques, designed the deletion mutant, produced recombinant proteins, participated in the sequence alignment analysis, standardized the IF/FISH assays and has been involved in drafting the manuscript. AMP participated in the production of recombinant proteins, performed in vitro binding assays and has also been involved in drafting the manuscript. RCVS and CEM obtained native protein extracts and performed

**Competitor.** Supershift assays were done using full-length recombinant LaTRF (10 μg) or native nuclear extracts from 10^8 parasites in the presence of ~30 μg of anti-LaTRF serum in EMSA buffer containing labeled LaTEL as probe and both poly [dI-dC] [dI-dC] and poly [dA-dT] [dA-dT] as above described. These assays were also performed in the presence of 20 fold excess of non-labeled LaTEL and 100 fold excess of poly [dI-dC] [dI-dC] as described above.

Western blots and chromatin immunoprecipitation assays. JLSN helped MSS with the cloning strategies, IF/FISH experiments and designed the peptide used to generate anti-LaTRF serum. LHJ collaborated in outlining some experimental strategies and has been involved in the manuscript revision contributing with important intellectual content. MNC coordinated and designed most of the experiments as well as the strategies used in the manuscript, has mentored MSS, AMP, RCVS and CEM, who have also contributed during discussions of the results. MNC critically read and reviewed the manuscript for its publication. All authors read and approved the final manuscript.

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