Developmental Regulation of Spliced Leader RNA Gene in Leishmania donovani Amastigotes Is Mediated by Specific Polyadenylation

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Leishmania cycles between the insect vector and its mammalian host undergoing several important changes mediated by the stage-specific expression of a number of genes. Using a genomic differential screening approach, we isolated differentially expressed cosmid clones carrying several copies of the mini-exon gene. We report that the spliced leader (SL) RNA, essential for the maturation of all pre-mRNAs by trans-splicing, is developmentally regulated in Leishmania donovani amastigotes and that this regulation is rapidly induced upon parasite growth under acidic conditions. Stage-specific regulation of the SL RNA is associated with the expression of a larger ~170-nucleotide transcript that bears an additional 15-nucleotide sequence at its 3'-end and is polyadenylated in contrast to the mature SL RNA. The poly(A)+ SL RNA represents 12–16% of the total SL transcript synthesized in amastigotes and is 2.5- to 3-fold more stable than the poly(A)− transcript. The poly(A)+ SL transcript is synthesized specifically from one class of the genomic mini-exon copies. Polyadenylation of the SL RNA may control the levels of the SL mature transcript under amastigote growth and may represent an additional step in the gene regulation process during parasite differentiation.

Leishmania is a protozoan parasite that infects and replicates within mammalian macrophages, thereby causing a wide spectrum of diseases in humans. The parasite’s life cycle comprises two stages involving two different hosts: the flagellated promastigote stage, which is present in the gut of the sand fly vector, and the aflagellated non-motile amastigote present in the gut of the sand fly. Leishmania donovani is developmentally regulated in the amastigote stage. This regulation involves a specific polyadenylation of a longer SL transcript, which seems to be synthesized specifically from one class of the genomic mini-exon repeats. This is the first time where polyadenylation of a normally non-polyadenylated RNA is reported as part of a putative regulatory mechanism aiming at controlling gene expression in a specific stage of Leishmania’s life cycle. Specific polyadenylation of the SL RNA may represent an additional step in the gene regulation process during Leishmania differentiation into the amastigote form.

EXPERIMENTAL PROCEDURES

Parasite Strains and Culture Media—L. donovani donovani Suda-

nese 1S2D and L. donovani infantum LEM1317 (from Dr. Bastien, Montpellier, France) strains were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated calf serum and 5 μg/ml he- min. Promastigotes were cultured in RPMI at pH 7.0 and 25 °C. To induce axenic differentiation we used a slightly modified protocol published by Joshi et al. (20) where stationary phase parasites were transferred into RPMI medium supplemented with 20% calf serum and MES
to maintain the pH at 5.0 and grown for 5 days at 25 °C before being shifted to 37 °C at a 5% CO₂ atmosphere for an additional 24 h. 

Amastigotes were also isolated from the spleens of gold Syrian hamsters infected intraperitoneally with 5 × 10⁵ L. donovani 1S2D amastigotes as described (21).

**Nucleic Acid Preparations and Analyses—Total RNA of L. donovani promastigotes and amastigotes was isolated using the guanidinium isothiocyanate method with TRIzol (Life Technologies, Inc.). cDNAs corresponding to all poly(A)⁺ mRNAs transcripts were synthesized using an oligo(dT)₁₇ primer and the Superscript II reverse transcriptase (Life Technologies, Inc.).** Southern and Northern blot hybridizations were performed following standard procedures (22). The two probes used for these studies correspond to the intron sequence of the spliced leader RNA gene generated by PCR using P1 (5'-ATTGGTATGCGAACTTCG-3') and P2 (5'-CGGCTACGGAGGCCGCCGGC-3') primers (see Fig. 8) and to the whole mini-exon repeat, as part of the 434-bp Neo fragment. Neomycin phosphotransferase (neo) and hygromycin phosphotransferase (hyg) specific probes were made by PCR and used for determining the vector copy number in the transfectants. The L. donovani infantum LEM1317 cosmid library was prepared by ligating size-selected Sau3AI partial digest products of genomic DNA with the shuttle vector cL-HYG (23), cleaved by BamHI, and packaged using the Gigapack Gold II kit (Stratagene). The library was gridded onto nylon membranes at the Toronto Genome Center (Hospital for Sick Children) to generate high density filters of 92 × 92 cm. High density filters of the same series were hybridized independently to L. donovani amastigote and promastigote ³²P-labeled cDNA probes.

**Recombinant DNA Constructs and Transfections—Constructs pSPneo- miniexIA, pSLneo- miniexIB, and pSLneo- miniexIC bearing the three different mini-exon genomic copies of L. infantum LEM1317 were made as follows. The 424-bp Neo fragments (copies B and C) from cosmid 3H4 (carrying L. donovani genes) were subcloned into the Neo site of pSL1180 (Amerham Pharmacia Biotech), and the SmaI-BamHI line expression cassette (24) from vector pSP72tRNA was introduced into EcoRV-BamHI sites of pSLneo- miniexIB and pSLneo- miniexIC, respectively. The 434-bp Neo fragment (copy A) was subcloned into the BamHI site of pSP72tRNA expression vector following a Klonein polynucleotide treatment. Expression of the neo gene in pSP72tRNA and pSLneo- is driven by the intergenic region of the α-tubulin gene (represented here by α) (25). The cDNAs of the poly(A)⁺ and poly(A)⁻ SL transcripts (see below) were amplified by RT-PCR using P1 (see Fig. 8) and an oligo(dT)₁₉ as primers and subcloned into pCR™ 2.1 (Invitrogen) to generate pSL-RNA(α)⁺ and pSL-RNA(α)⁻ vectors, respectively. The nucleotide sequence of the different genomic mini-exon repeats of L. donovani 1S2D and L. infantum LEM1317 and of the cDNAs was determined by an ABI Prism 377 DNA automated sequencer. 10-20 µg of plasmid or cosmid DNAs were used for transfections into L. donovani by electroporation as described previously (24).

**Nuclear Run-on and RNA Stability Assays—Nuclear run-on assays were performed essentially as described by Quijada et al. (3) using nuclei freshly isolated from L. donovani exponential phase promastigotes and amastigote cultures. To look for RNA stability, late-log phase promastigotes were submitted to growth conditions mimicking those found in the phagolysosomes at pH 5.0 for 5 days followed by 1–2 h of incubation at 37 °C in the presence of 5% CO₂ prior to the addition of 10 µg/ml actinomycin D (Sigma). Cells were harvested by centrifugation, and total RNA was extracted at different time points of actinomycin D treatment: 0, 30 min, 1, 2, 4, and 8 h. Total RNA samples were subjected to Northern blot analysis using the intron sequence of the mini-exon gene as a probe. Quantitation of the SL transcripts was done by densitometric analysis using a PhosphorImager with the ImageQuant 3.1 software.

**Mapping of the 3'-End of Poly(A)⁺ and Poly(A)⁻ SL RNAs—**To map the 3'-end of the polyadenylated SL RNA, a double-stranded cDNA was synthesized from axenic amastigotes using P1 and an oligo(dT)₁₇ as primers. 3'-RACE studies were used to map the 3'-end of the nonpolyadenylated SL transcript. A poly(A) tail was added at the 3'-end of this transcript following incubation of 10 µg of total L. donovani promastigote RNA with 5 units of poly(A) polymerase (Life Technologies, Inc.) according to the manufacturer's recommendations. RT-PCR reactions were carried out using the above set of primers. The PCR products were then gel-extracted, further purified using phenol/chloroform extraction, subcloned into the pCR™ 2.1 vector, and sequenced.

**RESULTS**

**Differential Expression of the SL RNA Gene in L. donovani Amastigotes—**To identify genes that are developmentally regulated, we developed a genomic differential screening approach where high density filters, each covering approximately one parasite genome, were used. A genomic cosmid library made from L. donovani infantum strain LEM1317 was gridded onto high density filters (1536 cosmid clones/filter), which were hybridized with ³²P-labeled cDNA probes derived from promastigote and amastigote RNAs. Comparative analysis of the hybridization signal intensity between the promastigote and amastigote cDNA probes in four series of high density filters allowed the identification of a number of cosmid clones that were much more expressed in amastigotes. Normalization of the amount of bacteria layered at each position was obtained by hybridization with a vector-specific probe (hyg). Among a large number of clones selected on the basis of their higher expression in amastigotes, 10 were related. Partial sequence analysis of these clones indicated that we were dealing with the spliced leader RNA gene locus. From those 10, cosmid 3H4 was chosen for further analysis (Fig. 1A). Southern blot differential hybridization of cosmid 3H4 using promastigote and amastigote cDNA probes revealed the presence of a 434-bp Neo genomic fragment hybridizing much stronger to the amastigote cDNA (Fig. 1B). The nucleotide sequence of this fragment confirmed the presence of the mini-exon gene repeat.

Total RNA preparations from promastigotes and axenic amastigotes were hybridized with a probe corresponding to the intron sequence of the L. infantum mini-exon repeat that we have sequenced. Two transcripts of different sizes were detected in the amastigote RNA preparation, one corresponding to the expected ~86-nt SL RNA and a second larger transcript of ~170 nt (Fig. 2A). In addition, a 2.5-fold increase in the 86-nt SL transcript was observed in the amastigote RNA preparation (Fig. 2A). To determine whether this developmental regulation of the SL transcript was in fact a physiological event and was not because of an artifact due to the axenic conditions used for parasite differentiation, we tested amastigotes freshly isolated from infected hamsters. As shown in Fig. 2B, a larger size SL transcript was detected in the RNA preparation from hamster-derived amastigotes similar to what was seen when using the...
axenic amastigotes. The larger SL transcript was estimated, by densitometric analysis using PhosphorImager scanning of several independent Northern blots, to represent 12–16% of the total SL RNA synthesized within the amastigote cell.

**Stage-specific Expression of the SL RNA Is Induced upon Growth at Acidic Conditions**—During their life cycle *Leishmania* are exposed to temperature and pH changes that trigger stage-specific differentiation (26). Elevated temperature or acidic pH has been associated with the induced expression of a number of genes (21, 27, 28). To assess the role of pH or of temperature in the differential regulation of the SL RNA, different culture conditions were tested. *L. donovani* promastigotes were subjected to a pH shift from pH 7.0 to 5.0 for 5 days at 25 °C or to a temperature shift at 37 °C in a 5% CO2 environment. The bottom panel represents an ethidium bromide staining of the RNA samples loaded on agarose gel.

**Differential Regulation of the SL RNA Is Because of a Specific Polyadenylation in Amastigotes**—We hypothesized that the larger 170-nt SL RNA present in amastigotes might be the result of an alternative 3'-end formation or of a post-transcriptional modification of the transcript. Several previous studies have reported that the kinetoplastid SL RNA, as for most of the SL RNAs in nematodes (29), is not polyadenylated and that it is constitutively expressed throughout the developmental stages of these parasites (30, 31). However, the SL transcript has been detected within the poly(A)+ RNA fraction of *T. brucei* bloodstream forms (32). To test whether polyadenylated SL RNA could also be found in *Leishmania*, we first synthesized cDNAs from total RNA of *L. donovani* promastigotes and axenic amastigotes using and oligo(dT) primer, allowing only the polyadenylated RNAs to be reverse transcribed, and hybridized them with the mini-exon repeat as a probe. Because of the presence of the 39-nt spliced leader at the 5'-ends of all pre-mRNAs, a hybridization smear corresponding to all polyadenylated transcripts was observed in both promastigote and amastigote cDNAs (Fig. 4A). A cDNA fragment corresponding to the SL RNA gene was found exclusively in the amastigote cDNA preparation, strongly suggesting that polyadenylated forms of the SL RNA could indeed be present in *Leishmania* amastigotes (Fig. 4A). To verify whether this poly(A)+ SL transcript was also found in *L. donovani* amastigotes isolated from infected hamsters, similar experiments were performed, and...
they gave rise to identical results (data not shown). The presence of the poly(A)$^+$ SL transcript in amastigotes was confirmed by RT-PCR studies where a cDNA synthesized from a poly(A)$^+$ fraction was subjected to PCR amplification using a combination of P1 (see Fig. 8) and oligo(dT) primers to amplify part of the SL transcript that lacks the common 39-nt spliced leader. A ~130-bp cDNA fragment amplified by PCR and corresponding to the poly(A)$^+$ SL RNA was detected by hybridization only in the amastigote poly(A)$^+$ fraction (Fig. 4B). PCR products hybriding to the intron probe were extracted from the agarose gel and subcloned into vector pCR TM 2.1. Sequence analysis definitively confirmed the poly(A)$^+$ addition in the SL transcript expressed specifically in the amastigote stage (Fig. 5A).

To compare the 3′-end of the poly(A)$^+$ and poly(A)$^-$ SL transcripts, we also mapped the 3′-end of the normally non-polyadenylated transcript in our L. donovani 1S2D strain by a 3′-RACE assay. The L. donovani poly(A)$^+$ SL RNA ended at position 86 (Fig. 5A), slightly different from what has already been published for other Leishmania species where the 3′-end was mapped at nucleotide 89 (31) or nucleotide 96 (33). Sequence comparison between the cDNAs corresponding to the polyadenylated and non-polyadenylated SL transcripts revealed a significant difference at their 3′-ends. Indeed, the poly(A)$^+$ SL RNA contained 15 additional nucleotides, ending by four Ts part of the 8T-track sequence (see Fig. 8) in comparison with the poly(A)$^-$ transcript (Fig. 5A). A long poly(A) tail of at least 46 As was added immediately after the four Ts. Considering that the poly(A)$^+$ SL RNA is ~170 nt long, the poly(A) tail could include as much as 80 As. Comparison of the predicted secondary structures of the poly(A)$^+$ and poly(A)$^-$ SL RNAs using the SQUIGGLES program did not suggest a significant overall change in the free energy required for structure formation ($\Delta G$), but it revealed the presence of a third stem loop structure at the 3′-end of the SL poly(A)$^+$ RNA (Fig. 5B), which could possibly be a substrate for RNA binding proteins.

Polyadenylation of SL RNA Increases Its Stability—The effect of pH on the stability and accumulation of the SL transcripts was addressed. We first evaluated, by run-on experiments, whether the increase in poly(A)$^+$ SL RNA accumulation or the presence of the poly(A)$^+$ SL transcript in amastigotes (see Figs. 2 and 3) was because of higher SL RNA synthesis rates in this parasite stage. Nuclei were freshly isolated from promastigotes, and axenic amastigotes and radiolabeled nascent RNAs were extracted and used to probe the mini-exon and control DNA sequences. No differences were observed in the overall synthesis of SL RNA between promastigotes and amastigotes (Fig. 6), suggesting that the regulation occurs post-transcriptionally. Although secondary structure predictions did not suggest that the addition of the 15 nucleotides at the 3′-end of the larger SL RNA should increase its stability significantly (see Fig. 5B), it is, however, known that polyadenylation in general increases message stability (for review see Ref. 34). We therefore looked to see whether the poly(A)$^+$ SL RNA was more stable compared with the poly(A)$^-$ transcript. Because we showed that the maximum accumulation of the polyadenylated SL RNA is observed in cells maintained at pH 5.0 (Fig. 3), we evaluated the stability of the SL transcripts under these growth conditions. In these assays, L. donovani promastigotes were transferred from pH 5.0 and 25 °C to 37 °C for 1 h to adapt in these conditions prior to the addition of 10 μg/ml actinomycin D, an inhibitor of RNA synthesis. Total RNA samples were extracted at different time points following the addition of actinomycin D to the culture medium and ana-
lyzed by Northern blot using the spliced leader intron as a probe. The relative amount of the poly(A)$^+$ and poly(A)$^-$ SL transcripts present in the cell at each time point was compared with the RNA levels present at time 0. Quantitative evaluation of the SL RNA levels obtained by densitometric analysis of radiolabeled membranes using a PhosphorImager demonstrated that the poly(A)$^+$ SL RNA was 2.5–3-fold more stable compared with the non-polyadenylated transcript (Fig. 7, A and B). Similar results were obtained from two more independent experiments. The small shortening of the poly(A)$^+$ SL RNA observed over time may correspond to differences in RNA migration in that part of the gel (not shown). In light of these results, the half-lives were established at 4 h for the poly(A)$^+$ SL RNA and at 2 h for the poly(A)$^-$ transcript.

**Stage-specific Polyadenylation of One Class of the Mini-exon Genomic Copies**—To examine whether differences within the non-transcribed spacer of the mini-exon genomic copies could account for the formation of the new 3′-end of the poly(A)$^+$ SL RNA, the nucleotide sequence of several copies was determined. Several ~434-bp NcoI copies derived from cosmid 3H4 were subcloned into pSPneoeo and PSLneoeo expression vectors, and their nucleotide sequence was compared. By compiling the sequencing data from a large number of clones (~20), we classified the mini-exon gene repeats into three classes (A–C). The alignment of the nucleotide sequences of these three different copies along with a previously published copy derived from another L. infantum strain is presented in Fig. 8. As expected, the 39-nt spliced leader was highly conserved, whereas three nucleotide changes and one short deletion were observed in the intron sequence of the two L. infantum strains. However, considerable differences were noted within the non-transcribed spacers of the two L. infantum strains (see Fig. 8). Analysis of the three copies of the LEM1317 strain showed only one difference located between nucleotides 140 and 150. Copy A indeed contains a 10-bp GC-rich stretch, which is absent in copies B and C, where only a single base pair mismatch from a T to C at position 150 distinguishes these two copies (Fig. 8).

We tested whether one of these genomic copies was associated with the larger ~170-nt SL transcript. Accordingly, we made the appropriate constructs to overexpress each one of the three mini-exon gene repeats (A–C) in L. donovani (Fig. 9A). We also transfected cosmid 3H4, which contains the three different classes of the mini-exon repeats. Stable transfectants were subjected to differentiation from promastigotes to axenic amastigotes. RNA samples from wild type and transfectants were analyzed by Northern blot using a probe corresponding to the spliced leader intron sequence. A significant increase in the poly(A)$^+$ SL transcript was detected only in the transfectants expressing copy A or cosmid 3H4, respectively (Fig. 9B). No differences between the transfectants carrying copy B or C and the wild-type strain were observed, suggesting a certain specificity of the polyadenylation process. To ascertain these results we compared the copy number of the expression vectors in the transfectants by hybridization studies using neo and hyg genes as probes. No significant differences in the copy number of the three mini-exon repeats were noted between transfectants (Fig. 9C), thus supporting further the implication of copy A in the formation of the longer SL transcript that becomes polyadenylated. By random sequencing of a large number of clones derived from cosmid 3H4 we roughly estimated that each class is equally represented in this cosmid.

**DISCUSSION**

Using a genomic differential screening approach based on the hybridization of high density filters with promastigote and amastigote cDNA probes, we have isolated a number of differentially expressed cosmids clones that contained several mini-exon gene repeats. In kinetoplastida, the SL RNA is encoded by a cluster of ~150–200 tandemly repeated copies per genome. Each repeat comprises a highly conserved 39-bp exon sequence, a moderately variable (55–101 bp) intron sequence, and a non-transcribed highly variable spacer between different genus and
FIG. 8. Sequence alignment of the three L. donovani infantum LEM1517 mini-exon gene copies. The ~434-bp Nco1 fragments derived from cosmid 3H4 were sequenced, and copies A, B, and C, which can be found under the accession numbers AF097653, AF097654, and AF097655, respectively, were aligned together with the mini-exon repeat of another L. donovani infantum MHOM 80 strain. The 39-nucleotide spliced leader is underlined. Open boxes indicate the differences depicted between the nucleotide sequences of the mini-exon gene repeats. Primers P1 and P2 used for PCR and RT-PCR studies are also indicated.

species (35–38). The spliced leader, a common 39-nucleotide sequence, is transferred via trans-splicing from the precursor SL RNA to the 5'-end of all trypanosomatid pre-mRNAs.

We have shown that stage-specific regulation of the SL RNA in both L. donovani and L. infantum is mediated by a specific polyadenylation process. Upon growth of the parasite at acidic pH, 12–16% of the total SL RNA becomes polyadenylated, giving rise to a larger 170-nt transcript in addition to the 86-nt mature SL RNA (Figs. 2, 3, and 5A). A temperature shift alone (25–37 °C) was not capable of inducing the expression of the polyadenylated SL RNA or of increasing the amount of the non-polyadenylated transcript (Fig. 3). Several reports have confirmed the constitutive expression of the SL RNA in all developmental stages of trypanosomes and Leishmania, and it is generally assumed that the SL RNA is not polyadenylated (30, 31). Similarly, spliced leader RNAs in the nematode Caenorhabditis elegans are normally not polyadenylated although one case has been reported (29). Polyadenylated forms of the SL transcript have been identified in poly(A) 1 RNA fractions from short stumpy bloodstream forms of T. brucei, however (32).

Mapping of the 3'-end of the poly(A) 1 and poly(A) 1 SL RNAs revealed that the poly(A) 1 SL RNA was longer by 15 nucleotides compared with the 86-nt non-polyadenylated SL RNA 1 (see Fig. 9). One important factor determined the site of polyadenylation in Leishmania is the positioning of a functional splice acceptor site immediately downstream as polyadenylation and trans-splicing seem to be coupled in this organism (17). Accurate polyadenylation in trypanosomatids is often determined by pyrimidine-rich elements in the intergenic regions (45–47). Polyadenylation sites are recognized by both the trans-splicing and polyadenylation machineries either sequentially or simultaneously (18). The additional 15-nt sequence part of the very 3'-end of the poly(A) 1 SL RNA is rich in pyrimidines (10 nucleotides of 15), and it contains an AG splice acceptor site immediately preceded by a pyrimidine tract (5'-GGCGCTCTTTT-3') (see Fig. 5A). This splice acceptor site may be recognized by splicing factors that could activate polyadenylation. This has been seen in higher eukaryotes (48).

Although polyadenylation has been reported in bacteria and some plastid mRNAs to accelerate transcript degradation (49, 50) it is generally assumed that in most eukaryotic systems the poly(A) tract protects mRNAs from rapid degradation (51–53). In addition, the poly(A) tail influences nuclear processing of the
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Fig. 9. Stage-specific polyadenylation of one class of the mini-exon genomic copies. A, schematic representation of the constructs used for the episomal expression of the L. infantum LEM1317 mini-exon gene repeats (A–C) into L. donovani. The expression of neo in these vectors is driven from the intergenic region of the α-tubulin gene (IR-α). B, Northern blot hybridization of mini-exon recombinant transfectants (3H4, A, B, C) and wild-type cells subjected to an axenic differentiation with the spliced leader intron probe. Pro, promastigotes; Am, amastigotes. C, estimation of the mini-exon repeats copy number in the transfectants shown in A by hybridization studies. Densitometric analysis was carried out as described in Fig. 7B, and the quantitation of the hyg- or neo-containing vectors was based on comparison studies with single integration of the neo and hyg genes within the parasite genome.

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pre-mRNA, nucleocytoplasmic transport, and translation (34, 53–55). Polyadenylation of the SL RNA in Leishmania increases its stability by a factor of 2.5–3 as estimated from the actinomycin D data (Fig. 7). The presence of the 150-nucleotide sequence at the 3'-end of the poly(A) SL RNA does not seem to introduce any significant change in the AG (from -21.2 to -18.8 kcal/mol) required for RNA structure formation, but it allows the formation of a stem-loop structure at the 3'-end of the transcript (Fig. 5B). Stem-loop structures at the 3'-ends of many transcripts function as binding sites for regulatory proteins that could affect RNA transport, translation, and half-life (34). It is possible that the poly(A) SL RNA is exported into the cytoplasm as part of a regulatory mechanism to decrease the overall levels of SL RNA in the nucleus of the amastigote cells. Amastigotes replicate more slowly than promastigotes, and it is likely that less SL RNA is required for mRNA processing by trans-splicing. This is in line with our observation that even the poly(A) SL RNA accumulates in the amastigote stage. Indeed, a 2.5-fold increase in SL transcript levels was seen during differentiation of the parasite into the amastigote form (Figs. 2 and 3). The half-life of transcripts could change in response to nutrient levels, cell growth rates, and temperature shifts, among other things (56), and this could also be the case for the SL RNA under acidic stress. It is also possible that the polyadenylated SL transcript cannot be used as a donor of the 39-nt spliced leader sequence to 5'-end of all pre-mRNAs. As a consequence, this should decrease trans-splicing reactions in amastigotes by ~15%. It has been shown that L. tarentolae SL transcripts that were malformed by more than 1 nucleotide at their 3'-ends were unable to participate in trans-splicing or acquiring a proper cap 4 methylation (39).

During their life cycle Leishmania protozoans alternate from the promastigote to the amastigote stage, undergoing several morphological and biochemical changes that are monitored by the differential expression of a variety of genes. Previous studies have reported amastigote-specific regulation of a number of genes: hsp83 (27), hsp100 (57), histone H1 (58), ephc cysteine proteinases (59), A2 gene family (21), and the LmcDNA16 gene family (60). Our studies demonstrated that part of the spliced leader RNA in L. donovani amastigotes is polyadenylated. This polyadenylation is stage-regulated and may correspond to a general post-transcriptional mechanism operating upon parasite differentiation to control gene expression.

FIG. 9.
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