Amastin mRNA Abundance in Trypanosoma cruzi Is Controlled by a 3′-Untranslated Region Position-dependent cis-Element and an Untranslated Region-binding Protein

(Received for publication, July 16, 1999, and in revised form, December 3, 1999)

Bridget C. Coughlina, Santuza M. R. Teixeirab,§, Louis V. Kirchhoffc, and John E. Donelsonta**

From the Departments of bBiochemistry and dInternal Medicine, University of Iowa and the eDepartment of Veterans Affairs Medical Center, Iowa City, Iowa 52242

The genome of Trypanosoma cruzi contains tandem arrays of alternating genes encoding amastin and tuzin. Amastin is a surface glycoprotein abundantly expressed on the intracellular mammalian amastigote form of the protozoan parasite, and tuzin is a G-like protein. We demonstrated previously that the amastin-tuzin gene cluster is polycistronically transcribed to an equal extent in all parasite life cycle stages. The steady state level of amastin mRNA, however, is 68-fold more abundant in amastigotes than in epimastigotes. Here we show that the half-life of amastin mRNA is 7 times longer in amastigotes than in epimastigotes. Linker replacement experiments demonstrate that the middle one-third of the 630-nucleotide 3′-untranslated region (UTR) is responsible for the amastin mRNA up-regulation. This positive effect is dependent on the distance of the 3′-UTR segment from the stop codon and the polyadenylation site as well as on its orientation. A protein or protein complex more abundant in amastigotes than in epimastigotes binds to this minimally defined 3′-UTR segment and may be involved in its regulatory function.

Trypanosoma cruzi is the protozoan parasite that causes Chagas’ disease, a debilitating illness that is a major cause of morbidity and mortality in many parts of Latin America. During its life cycle, T. cruzi passes through three developmental stages. In their insect vectors, the parasites multiply as extracellular epimastigotes in the midgut, migrate to the hindgut, and then differentiate into nondividing trypomastigotes. These infective organisms are excreted in the feces after a blood meal. When they contaminate the puncture site or mucous membranes of a mammalian host, they can invade a variety of cell types. Once inside host cells, the trypomastigotes differentiate into amastigotes, which multiply in the cytoplasm. After many cycles of proliferation, the amastigotes differentiate into epimastigotes and enter the circulation when the host cell ruptures. These epimastigotes perpetuate either the infection or the life cycle when they invade other host cells or are ingested by insect vectors, respectively.

Since the intracellular amastigotes are at the core of the persistence of T. cruzi infection, we previously sought to identify genes that are preferentially expressed by amastigotes in order to develop a better understanding of the molecular biology of this parasite form. We identified two novel genes, encoding the proteins amastin and tuzin, which occur in alternating tandemly arranged pairs (1, 2). Amastin is an abundant glycoprotein on the surface of amastigotes. Less is understood about tuzin’s function or location, but it possesses sequence similarity to a heterotrimeric G-protein. Most tandem genes in T. cruzi and other trypanosomatids, such as Leishmania species and African trypanosomes, do not have recognizable upstream promoters and are transcribed into polycistronic precursor RNAs (3–6). The 5′ ends of the resulting mature monocistronic mRNAs are defined by the trans-splicing of a 39-nucleotide spliced leader (SL)1 sequence, and the 3′ ends of the mRNAs are generated by polyadenylation (3, 7–9). In general, polyadenylation of the upstream coding region in the precursor RNA is coupled with the SL trans-addition of the downstream coding region (10, 11). Although amastin and tuzin genes are present in alternating pairs and are transcribed at equal levels in amastigotes and epimastigotes, the steady state level of amastin mRNA in amastigotes is at least 68-fold higher than in epimastigotes (2). Tuzin mRNA, however, is at an equally low level in the two life cycle stages and is approximately as abundant as amastin mRNA in epimastigotes. Thus, post-transcriptional controls must confer this dramatic life cycle-specific gene regulation.

The abundance levels of several other life cycle-specific trypanosomatid mRNAs, such as the mRNAs encoding the Trypanosoma brucei procyclin and hexose transporters, and Leishmania gp63 and hsp83 have been shown to be dependent on sequences within their 3′-untranslated regions (UTRs) (4, 12–15). Moreover, our previous studies indicated that the amastin 3′-UTR is also necessary for its post-transcriptional up-regulation in amastigotes (1). There are numerous examples in other eukaryotic systems of cis-elements in 3′-UTRs of mRNAs that confer either stability, translatability, or precursor RNA processing signals (16–18), and it is thought that many of these cis-elements act in concert with trans-acting factors that are ultimately responsible for the fate of the RNA (19). In trypanosomatids, due to the absence of polymerase II promoters and heavy reliance on post-transcriptional control, there is perhaps even greater dependence on regulatory cis-acting RNA elements and cis-trans interactions. However, despite their inferred significance, very little is understood about the molecular mechanisms underlying these regulatory RNA

* This work was supported in part by National Institutes of Health Research Grants AI40591 and TW00998. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† Present address: Bioquímica e Imunologia, ICB-UFMG, Belo Horizonte, MG-Brazil 31270-010.
** To whom correspondence should be addressed: Dept. of Biochemistry, University of Iowa, Iowa City, IA 52242. Tel.: 319-335-7934; Fax: 319-353-4204; E-mail: john-donelson@uiowa.edu.

1 The abbreviations used are: SL, spliced leader; IR, intergenic region, nt, nucleotide; PCR, polymerase chain reaction; pre-mRNA, precursor mRNA; UTR, untranslated region; bp, base pair; PBS, phosphate-buffered saline; MOPS, 4-morpholinepropanesulfonic acid; GFP, green fluorescent protein.
sequences, and even less is known about their protein interactions.

We report here that three potentially linked mechanisms are responsible for the up-regulation of amastin mRNA in amastigotes. First, the half-life of the amastin mRNA is 7 times longer in amastigotes than epimastigotes. Second, positive regulatory elements occur within the central one-third of the amastin mRNA’s 630-bp 3′-UTR. This central portion of the amastin 3′-UTR is essential for up-regulation of a reporter gene, and the positive effect is rigidly dependent on the distance of the regulatory 3′-UTR segment from the stop codon and the polyadenylation site, as well as on its orientation. Third, a 30–36-kDa protein or protein complex in amastigotes, but not in epimastigote extracts, binds to the essential 3′-UTR regulatory cis-element.

**EXPERIMENTAL PROCEDURES**

*Parasite and Cell Cultures—* The Tulahén strain of *T. cruzi* was used for all experiments (20). Epimastigotes were maintained in logarithmic growth phase at 26 °C in supplemented liver digest-neutralized tryptose medium as described previously (21). Culture-derived amastigotes were obtained by infecting monolayers of the RA786 renal carcinoma cell line (22) grown at 37 °C in RPMI supplemented with 10% heat-inactivated fetal bovine serum, 5% l-glutamine, and 5% penicillin/streptomycin. Amastigotes were purified by centrifugation in metrizamide gradients as described previously (2).

*Actinomycin D Treatment and Northern Blot Analysis to Determine mRNA Half-life—* A total of 10⁹ parasites in 5 ml of liver digest-neutralized tryptose medium received 72 μg of actinomycin D in 8 μl of dimethyl sulfoxide and were allowed to incubate for various time periods. Time point 0 received no actinomycin D. After incubation for the appropriate time, cells were pelleted and washed in phosphate-buffered saline (PBS). Total RNA was harvested using the RNeasy kit (Qiagen). Either 40 μg of epimastigote RNA or 10 μg of amastigote RNA was subjected to electrophoresis in a 1.1% agarose/MOPS/formaldehyde gel. The gel was blotted to nitrocellulose, cross-linked, and hybridized at 42 °C with a radiolabeled amastin 3′-UTR probe generated with the Random Prime System (Roche Molecular Biochemicals). Following washing at 65 °C in 1× SSC, 0.1% SDS, radioactivity was measured using a Hewlett-Packard Instant Imager. The blots were then reprobed using a radiolabeled RNA fragment, and again the signal was quantitated to allow normalization for the amount of RNA present in each lane.

*Plasmid Constructs—* The sequences of the oligonucleotides used in this study are presented in Table I. Nucleotide numbering of the 3′-UTR is referenced from the amastin stop codon. All plasmids used in this study are presented in Table I. Nucleotide numbering of the 3′-UTR was amplified using oligonucleotides A₅ and A₆ that hybridize to regions immediately downstream from the h and h oligonucleotide hybridization sites (Fig. 2A).

This amplification generated a fragment designated A₂A₆. The A₂A₆ -UTR was amplified using oligonucleotides A₂ and A₆. The A₂A₆ -UTR and the amastin 3′-UTR were generated by PCR amplification of the TCR7 5′ and 3′ halves using oligonucleotides A₅02.3, S₂₀₈.₅, S₀.₅, and A₂₃₁.₃ (Table I). This amplification generated a fragment designated A₅1₂.₅. The A₅1₂.₅ fragment was positioned downstream from the luciferase coding region into the XhoI site of the pLCTCR7 plasmid and upstream of the full-length TCR7 3′-UTR to generate the four plasmids pLCTCR7/A₅1₂.₅(+1x), pLCTCR7/A₅1₂.₅(-1x), pLCTCR7/A₅1₂.₅(-2x), and pLCTCR7/A₅1₂.₅(-3x). The chimeric 3′-UTRs containing the TCR7 3′-UTR and the amastin 3′-UTR were generated by PCR amplification of the TCR7 3′-UTR’s 5′ and 3′ halves using oligonucleotides A₅02.3, S₂₀₈.₅, S₀.₅, and A₂₃₁.₃ (Table I). The 5′-half of the TCR7 3′-UTR, the central portion of the amastin 3′-UTR and the 3′ half of the TCR7 3′-UTR were then ligated and inserted into pGEM-luc. Since the amastin 3′-UTR fragment had blunt ends, it could be ligated in either orientation and in multiple copies.

To generate the plasmids that contained the amastin 3′-UTR element inserted within the TCR7 3′-UTR (plasmids pLCTCR7/A₅1₂.₅(+1x)/mid and pLCTCR7/A₅1₂.₅(-1x)/mid), oligonucleotides were generated to separately amplify the 3′ and 5′ portions of the TCR7 3′-UTR. The two halves of the TCR7 3′-UTR were then ligated to the A₅1₂.₅ fragment.

To provide templates for *in vitro* transcription from either a T3 or T7 polymerase promoter, the wild-type amastin 3′-UTR from the pGEM-luc construct was subcloned into the pBSK(+)/−. The linker mutants were subcloned into BamHI and KpnI sites of pBSK(+)/−, and the A₅1₂.₅ fragment was subcloned into the EcoRI site.

**DNA Transfections, Luciferase, and β-Galactosidase Assays—** Plasmid DNA used for transfection was prepared by alkaline lysis of host bacteria followed by column purification (Qiagen). Transfections of epimastigotes or amastigotes performed with either 15 or 50 μg of circular plasmid DNA. All test plasmids were co-transfected with an equal amount of the β-galactosidase encoding plasmid. Briefly, 10⁶ parasites were washed in 350 μl of PBS, containing 0.5 mM MgCl₂ and 0.1 mM CaCl₂. Cell suspensions were mixed with 50 μl of plasmid DNA, and after a 10-min incubation on ice, they were subjected to one pulse at 500 V and 450 microfarads in a 0.2-cm cuvette using a Gene Pulser (Bio-Rad). Ten minutes after transfection, amastigotes were transfected to 5 ml of liver digest-neutralized tryptose medium and incubated at 26 °C for 48 h. Amastigotes were transfected to a 25-cm² tissue culture flask containing 50% confluent RA786 cells with 5 ml of supplemented RPMI and were incubated at 37 °C and 5% CO₂ for 48 h. Luciferase and β-galactosidase assays, the cells were harvested, washed in PBS, transferred to 1.5-ml capped tubes, and centrifuged for 10 s at thermodynamically stable RNA secondary structures. The reconstructed 3′-UTR with its native 70 bp replaced by the synthetic linker was then ligated into pGEM-luc digested with Clal and SacI.
Differential Stabilities of Amastin mRNA in Amastigotes and Epimastigotes Account in Part for the Different Steady State Levels of the amastin mRNA in the Two Life Cycle Stages. The increase in amastin mRNA in amastigotes immediately following the addition of actinomycin D is probably due to a temporary stress-induced increase in total mRNA levels prior to terminating transcription. Similar temporary increases in mRNA abundance following actinomycin D addition have been documented elsewhere (9). This increase, however, does not preclude extrapolation of a relative mRNA decay rate, since decay values calculated from either the 0 or 10 min time point are not significantly different.

RESULTS

Differential Stabilities of Amastin mRNA in Amastigotes and Epimastigotes Account in Part for the Different Steady State Levels—We reported previously that the steady state level of amastin mRNA in T. cruzi TCR27 is edly higher level of amastin mRNA in amastigotes than in epimastigotes. To investigate the extent to which mRNA stability is responsible for this difference, the half-lives of the amastin mRNA in the two life cycle stages were assayed for luciferase activity as described previously (3). Assays for $\beta$-galactosidase activity were performed with a similar volume of lysate using the CLONTECH Laboratories system.

A 1:5 dilution (epimastigotes) or two aliquots of 20 $\mu l$ (amastigotes) were assayed for luciferase activity as described previously (3). Assays for $\beta$-galactosidase activity were performed with a similar volume of lysate using the CLONTECH Laboratories system.

Protein Extracts—Parasite protein extracts were generated by pelleting 10$^9$ cells, washing two times in PBS, and lysing in 20 $\mu l$ of Munro’s buffer (200 mM KCl, 0.1% Nonidet P-40). After a 10-min incubation on ice, 180 $\mu l$ of Munro’s buffer was added, and the cells were passed 10 times through a 23-gauge needle. Lysates were then centrifuged at maximum speed in a tabletop centrifuge for 10 min. The supernatant was removed, and protein concentration was determined using the CLONTECH Laboratories system.

Munro’s buffer (200 mM KCl, 0.1% Nonidet P-40). After a 10-min incubation on ice, 180 $\mu l$ of Munro’s buffer was added, and the cells were passed 10 times through a 23-gauge needle. Lysates were then centrifuged at maximum speed in a tabletop centrifuge for 10 min. The supernatant was removed, and protein concentration was determined using a standard Bradford assay. Mammalian cell extracts were generated similarly, with the exception that only 10$^5$ cells were used, 80 $\mu l$ of Munro’s buffer was added after initial lysis, and cells were passaged through a 100-$\mu l$ pipette tip.

In Vitro Transcribed RNAs—Plasmids were linearized at the polylinker sites just downstream or upstream from the cloned insert and transcribed with either T7 or T3 RNA polymerase to generate sense or antisense RNAs. Radiolabeled RNAs were synthesized in the presence of $\alpha$-32PUTP, using conditions recommended by Roche Molecular Biochemicals. The integrity of the transcribed product was assessed by electrophoresing either 10$^5$ cpm or 500 ng of RNA in an ethidium bromide-agarose/MOPS/formaldehyde gel, blotting to nitrocellulose, and performing conventional autoradiography. Alternatively, the size of the in vitro transcribed RNA was compared with that of RNA markers. The full-length amastin 3′-UTR and IR RNA probes contained the sequences immediately downstream from the luciferase stop codon to the end of the amastin/tuzin IR (1.3 kb). The A$_A$m domain of the amastin 3′-UTR contains the region flanked by these primers (210 bp).

RNase Protection Assay—In vitro transcribed RNA (10,000–65,000 cpm) was incubated with 40 $\mu g$ of protein from the appropriate cell extract. Nonradioactive RNA competitor, if needed, was added 10 min prior to the addition of the radiolabeled RNA. After incubation at room temperature for 20 min, the samples were cross-linked by exposure to an x-ray source of 3500 (× 100) $\mu$W at a distance of 4 cm. Samples were then hybridized with 10 units of RNase T1 for 10 min at room temperature followed by another 10-min incubation with heparin at 5 mg/ml. Samples were boiled in loading buffer and separated in a 5% stacking, 10% resolving Tris-glycine SDS-polyacrylamide gel at 100 V. The gel was dried onto Whatman paper and subsequently used to expose film.

Statistical Analysis—Prior to mRNA half-life determination, each time point was normalized to the relative hybridization signal from a ribosomal RNA probe to adjust for slight differences in the quantity of RNA in each lane. Half-lives for each experiment were determined by linear regressions drawn by the Microsoft Excel program. The mRNA half-lives are presented as the mean and S.D. of each experiment. Student’s unpaired $t$ test was employed to compare amastigote and epimastigote mRNA half-lives. Luciferase percentages are given as normalized averages of at least six independent samples assayed in duplicate. Efficiency of transfection was assessed by co-transfecting the $\beta$-galactosidase encoding plasmid with each test plasmid and normalizing to $\beta$-galactosidase activity. If the normalized values deviated from the control plasmid by more than 15%, that sample was eliminated from consideration. The luciferase value of the wild type 3′-UTRs was set at 100%, and the corresponding values obtained with the mutant 3′-UTRs were adjusted accordingly.

RESULTS

Post-transcriptional mRNA Regulation in T. cruzi

72 $\mu$g of actinomycin D/ml/10$^8$ parasites was determined to be sufficient to stop >90% of RNA synthesis (data not shown). To analyze the decay rates of the amastin mRNA, amastigotes and epimastigotes were incubated in the presence of this amount of actinomycin D, and total RNA was harvested at various time points. The amount of amastin mRNA was then determined by Northern blot analysis and quantitated on a Hewlett Packard Instant Imager (Fig. 1). After normalizing to the hybridization signal of a 240S rRNA probe to adjust for differences in the quantity of RNA loaded in each lane, the amastin mRNA half-life in amastigotes was found to be 74 ± 11 min. The decay rate in epimastigotes was much faster, with a half-life of only 11 ± 5 min. These results suggest that amastin mRNA is either stabilized in amastigotes and/or destabilized in epimastigotes. The approximately 7-fold difference in decay rates accounts in part for the dramatic 68-fold difference reported earlier for the steady state levels of the amastin mRNA in the two life cycle stages. The increase in amastin mRNA in amastigotes immediately following the addition of actinomycin D is probably due to a temporary stress-induced increase in total mRNA levels prior to terminating transcription. Similar temporary increases in mRNA abundance following actinomycin D addition have been documented elsewhere (9). This increase, however, does not preclude extrapolation of a relative mRNA decay rate, since decay values calculated from either the 0 or 10 min time point are not significantly different.

Cis-acting Elements in the Amastin 3′-UTR Are Responsible for the Stage-specific Expression of Amastin mRNA—Because of precedents in other eukaryotic cells as well as in other trypanosomatids, we suspected that cis-acting elements in the amastin/tuzin gene cluster could be responsible for the markedly higher level of amastin mRNA in amastigotes than in epimastigotes. To test this possibility, in an earlier study we PCR-amplified from a genomic clone of the gene cluster the region between the amastin stop codon and the start codon of the downstream tuzin gene. This segment was designated the amastin 3′-UTR and IR. This fragment was then inserted into an expression plasmid downstream from a cassette containing a T. cruzi rRNA gene promoter, the amastin gene's 5′-UTR and IR, and a luciferase reporter gene for expression in a transient transfection assay. The regulatory effects conferred by the amastin 3′-UTR and IR, as reflected by luciferase production in transiently transfected amastigotes and epimastigotes, was compared with the expression regulated by the 3′-UTR and IR of the constitutively expressed T. cruzi TCR27 gene (23, 24). This gene encodes a cytoskeleton protein whose mRNA level is the same in amastigotes and epimastigotes. Relative to the expression controlled by the TCR27 3′-UTR and IR, the amastin 3′-UTR and IR decreased luciferase activity 5-fold in epimastigotes and increased luciferase activity 7-fold in amastigotes (1). Thus, in this earlier work we documented that the amastin 3′-UTR and IR affect expression in both a positive and negative manner, depending on which developmental stage contains the reporter plasmid.

Comparison of six independently cloned amastin cDNAs revealed virtually no variability among the 630-bp 3′-UTRs. Consequently, since no 3′-UTR region was more highly conserved than another region, by comparison alone we could not predict which, if any, segment might affect the regulation observed in the initial transfection experiments. Therefore, to characterize further the role of the amastin 3′-UTR and IR in the differential expression of amastin mRNAs, plasmids were constructed that contain truncations in either the 3′-UTR or IR or both (Fig. 2). One mutated 3′-UTR contains a 250-bp deletion of the 3′ half. Another plasmid was engineered to have a 100-bp deletion in the IR. These altered versions of the amastin 3′-UTR and IR
were transiently expressed in epimastigotes and amastigotes, and the relative luciferase values were determined. Compared with luciferase expression under the control of the full-length 3'-UTR and IR, the truncated 3'-UTR resulted in a small increase in the amount of luciferase activity in epimastigotes (119%) and a dramatic decrease in expression in amastigotes (12%). These results suggest that the deleted 3'-UTR contributes to the different steady state levels of amastin mRNA in epimastigotes and amastigotes. Deletions of the IR had no effect on luciferase activity in epimastigotes (102%) but decreased it 5-fold (20%) in amastigotes compared with that induced by the native sequence.

To identify more completely the 3'-UTR cis-elements responsible for the observed differential regulation, a more refined linker scanning mutagenesis approach rather than more deletion analyses was adopted. Contiguous 70-bp segments of the 630-bp 3'-UTR were replaced with a double-stranded, 70-bp synthetic oligonucleotide. This synthetic linker maintained the relative nucleotide base composition of the amastin 3'-UTR but was designed to contain no known RNA regulatory motifs or thermodynamically stable secondary structures.

The nine insertions of the 70-bp linker constructs span the entire 630-bp 3'-UTR and, similar to the plasmids shown in Fig. 2, are downstream from a luciferase gene under the control of an rRNA promoter and an amastin SL addition site (Fig. 3). The linker plasmids were transiently expressed in epimastigotes and amastigotes, and luciferase activity was determined and adjusted for the efficiency of transfection.

None of the replacement regions had a dramatic effect on luciferase expression in epimastigotes, although when the linker was at the 5’-most position (i.e. pLRA:h9), there was an increase in luciferase activity to 133 ± 11% above that of the uninterrupted 3'-UTR (100 ± 11%). One interpretation of this result is that the linker replaced a modest epimastigote-specific negative regulatory cis-element, thus relieving the repressive effects of the native sequence. When the linker was moved in

---

**FIG. 1. Amastin mRNA decay in amastigotes and epimastigotes.** Ten μg of total amastigote RNA (A) or 40 μg of epimastigote RNA (B) were harvested at the indicated times after actinomycin D addition and were separated according to methods outlined under “Experimental Procedures.” The Northern blot membranes were probed with the amastin 3'-UTR. Blots depicted in A and B were then reprobed with a rRNA fragment to assess the relative amount of RNA loaded per lane. C, graphical representation of the amastin mRNA half-life quantitations in amastigotes and epimastigotes from five independent experiments, as determined by radiodensitometry. RDU, relative density units.
the 3’ direction, as in constructs pLRA:h8 through pLRA:h1, there was minimal to no effect on reporter activity in epimastigotes, presumably because the negative regulating cis-element is confined to the h6 position.

In contrast to the transfections in epimastigotes, replacements of several segments of the 3’-UTR had a major effect on luciferase activity in transfected amastigotes. The regions replaced in plasmids pLRA:h4 through pLRA:h6 were critical for maximal luciferase expression in transiently transfected amastigotes. When the linker was moved from the h7 to the h6 position, luciferase activity diminished from 93 to 32%. When the linker was in the h4 position, only 11% of the luciferase activity was obtained compared with that induced by the full-length uninterrupted 3’-UTR. When the linker was positioned 70 bp in the 3’ direction, to generate pLRA:h3, luciferase activity was restored to 85% of wild type activity. These data support the hypothesis that an amastigote-specific positive cis-acting regulator of the amastin RNA is located between nucleotides 215 and 418 (regions h6–h4) of the 3’-UTR, and at its 3’ end either by the amastin 3’-UTR and IR or a truncated 3’-UTR and IR (orange boxes). T. cruzi epimastigotes or amastigotes were transfected with 15 or 55 μg, respectively, of each plasmid, and the luciferase assays were performed 48 h later. All test plasmids were co-transfected with a plasmid containing a β-galactosidase reporter gene flanked by the processing sequences of the constitutively expressed TCR27 gene. After normalizing for efficiency of transfection using β-galactosidase activity, luciferase activity from plasmid pLRA was set to 100%, and the relative luciferase values from plasmids pLRA:Δ250UTR and pLRA:Δ100IR were adjusted accordingly. For each plasmid, the data represent n = 10 and the S. E. are <±18%.

Up-regulatory Cis-acting Elements in the Amastin 3’-UTR Are Position-dependent but Contextually Independent—Since the h6–h4 region was necessary for maximal reporter gene expression in amastigotes, additional plasmids were generated to determine if this region is sufficient to confer the amastigote-specific up-regulation without the influence of the flanking 5’ and 3’ segments of the amastin 3’-UTR or IR. Plasmids pLRT and pLTCR27 were used as the parent plasmids for this analysis (Fig. 4A). These plasmids possess the 3’-UTR of the constitutively expressed T. cruzi TCR27 gene (23, 24). pLTCR27 also contains the IR downstream of the amastin gene, whereas pLRT does not have a corresponding IR. Compared with pLRA, which contains the amatin 3’-UTR plus IR, these plasmids direct less luciferase activity when transfected into amastigotes (14% for pLRT and 30% for pLTCR27 versus 100% for pLRA). This lowered amount of luciferase reflects the up-regulation in amastigotes provided by the amastin 3’-UTR plus IR, as described previously (1, 2). The 203-bp h6–h4 region was PCR-amplified using primers A9 and A6, which hybridize just outside the h6–h4 domain (see Fig. 2A). The resulting A9:A6 fragment was then subcloned into pLTCR27 immediately downstream from the luciferase stop codon and upstream from the TCR27 3’-UTR and amastin IR, generating a plasmid designated pLTCR27/A9:A6 (+1x) (Fig. 4A). To explore potential additive effects of the sequences in the h6–h4 region, this fragment was also inserted as three tandem repeats to yield plasmid pLTCR27/A9:A6 (+3x). To account for specificity, control plasmids were constructed that contain one or two copies of the h6–h4 region inserted in the opposite orientation, i.e.
the expression of the co-transfected 2. Luciferase activity was assessed 48 h after transient transfection into either epimastigotes or amastigotes, and the values were normalized to

or absence of the A2A6 domain and with or without the amastin IR.

are inserted into the ___, in plasmid h8 it replaces the sequence between __. The colored rectangles denote the same sequence elements as in Fig. 2B. Luciferase activity was assessed 48 h after transient transfection into either epimastigotes or amastigotes, and the values were normalized to the expression of the co-transfected β-galactosidase control plasmid. The data represent n > 15 and the S.E. are < ±11%.

Fig. 3. Identification of sequences within the amastin gene 3′-UTR that facilitate expression of a luciferase reporter gene in epimastigotes and amastigotes. Parent plasmid pLRA shown in Fig. 2 was mutated so that contiguous 70-bp regions were replaced by a synthetic 70-mer duplex oligonucleotide (patterned rectangles), which contains the same relative nucleotide ratios as those found in the endogenous amastin 3′-UTR. Insertion sites of the 70-mer are noted in Fig. 2A. For example, in plasmid h8 it replaces the sequence between __. The expression of the co-transfected β-galactosidase control plasmid. The data represent n > 15 and the S.E. are < ±11%.

A

| % Luciferase activity | Amastigote |
|----------------------|------------|
| pLRA                 | 100        |
| pLRT                 | 14         |
| pLTCR27              | 30         |
| pLTCR27/A2A6 +1x     | 32         |
| pLTCR27/A2A6 +3x     | 52         |
| pLTCR27/A2A6 -1x     | 27         |
| pLTCR27/A2A6 -2x     | 26         |
| pLRT/A2A6 +1x/mid    | 65         |
| pLTCR27/A2A6 +1x/mid | 92         |
| pLTCR27/A2A6 -1x/mid | 35         |

B

When epimastigotes were transfected with the plasmids, none of the resulting normalized luciferase values were statistically different from the control value, indicating that in epimastigotes luciferase expression was independent of the presence, orientation, and copy number of the A2A6 fragment (data not shown). However, a different pattern of expression was observed when amastigotes were transfected with these plasmids. An increase in luciferase activity was observed when three copies of A2A6 were present in the positive orientation (52% of pLRA for pLTCR27/A2A6 (+3x)). Significant up-regulation was not seen, however, when only one copy of A2A6 was present (32% for pLTCR27/A2A6 (+1x) versus 30% for pLTCR27) or when it was ligated in the opposite orientation as a single or double insert (27 and 26% for pLTCR27/A2A6 (−1x) and pLTCR27/A2A6 (−2x), respectively).

We reasoned that the 52% level of luciferase expression seen in the plasmid containing three copies of A2A6 might be the result of the middle copy being approximately the same distance from the luciferase stop codon as it is in the native amastin 3′-UTR. In the native amastin 3′-UTR, the 5′ end of the A2A6 fragment is 250 nt from the stop codon. Since the middle copy of A2A6 is similarly located in pLTCR27/A2A6 (+3x), and because only this plasmid caused up-regulation, we inferred that the positive regulating cis-element in the h6–h4 segment may be contextually and/or positionally dependent.

To address this possibility as well as the possibility that the A2A6 interacts with the amastin IR sequences, we constructed a plasmid in which the amastin 3′-UTR fragment is located within the TCR27 3′-UTR instead of upstream from it (Fig. 4B). Insertion of the cis-element into this site placed it 290 nt from the stop codon and 180 nt from the polyadenylation site, similar to that of the native amastin 3′-UTR. Fig. 4B shows that when these distances are conserved, up-regulation is clearly evident despite the presence of flanking TCR27 3′-UTR sequences. The luciferase activity produced by pLRT/A2A6 (+1x/mid) was two-thirds the activity of the full-length amastin 3′-UTR and IR (65 versus 100%). When the insert was positioned at the correct distance as well as with the amastin downstream IR (pLTCR27/A2A6 (+1x/mid)), there was full recovery of reporter activity (92%). In contrast, when the insert was in the reverse orientation, no effect was seen (pLTCR27/A2A6 (−1x/mid)). Thus, within the 9% S.D. of the experiments, pLRA and pLTCR27/A2A6 (+1x/mid) give the same amount of luciferase activity in amastigotes. As before, transfection of these plasmids into epimastigotes did not result in a change in luciferase activity (not shown). These results further support the concept that the positive regulatory sequences in A2A6 (the
h6–h4 domain) act in an orientation- and position-dependent manner in amastigotes as well as interact with the amastin IR sequences.

The luciferase data from the transient transfections shown in Figs. 2–4 indicate that sequences of the amastin 3'-UTR and IR contain major components for the post-transcriptional regulation of amastin gene expression. However, it is not clear from these transient transfections if these sequences affect pre-mRNA processing/mRNA stability or, alternatively, cause differential mRNA translation. To distinguish between these two possibilities, we used two cell lines in which the gene for the green fluorescent protein (GFP) was stably integrated into the genome. In both cases, the region upstream of the GFP gene contains the T. cruzi rRNA promoter and the amastin spliced leader site. In one case, the region downstream of the GFP gene is the wild type amastin 3'-UTR and IR (25). In the other case, the region downstream is the chimeric amastin/TCR27 3'-UTR followed by the amastin IR and was derived from pLTCR27/A2A6(+1x)/mid (Fig. 4B). The left panel of Fig. 5 shows that in the cell line bearing the GFP gene with the wild type amastin 3'-UTR and IR, the differential expression of GFP mRNA is similar to that of native amastin mRNA. In amastigotes, GFP mRNA expression is approximately 79-fold higher than the GFP mRNA level in epimastigotes. Since the elements common to the GFP gene and the amastin gene are the amastin gene's UTR and IR sequences, we conclude that these sequences regulate the mRNA abundance of the gene to which they are fused. The right panel shows that chimeric amastin/TCR27 3'-UTR that was sufficient to up-regulate luciferase expression in Fig. 4B (pLTCR27/A2A6(+1x)/mid) also up-regulated GFP mRNA in amastigotes 40-fold compared with epimastigotes. Although this chimeric region did not up-regulate GFP mRNA quite as much as the wild type amastin 3'-UTR and IR, the 40-fold difference is still consistent with the 68-fold difference in expression of the endogenous amastin gene. These results with the stably transfected cell lines strongly suggest that the differential luciferase expression shown in Figs. 2–4 is due to differences in the reporter's mRNA levels and not due to changes in translation of the mRNA.

An Amastigote-specific Protein Binds to the Amastin 3'-UTR—Previous studies using cycloheximide inhibition of protein synthesis suggested that a labile protein factor(s) stabilizes amastin mRNA in amastigotes and destabilizes tuzin mRNA in both epimastigotes and amastigotes (Ref. 2 and data not shown). To investigate this possibility, we used RNA gel shift and RNase protection assays to determine if an amastigote-specific protein interacts with the amastin 3'-UTR. T. cruzi epimastigote or amastigote protein extracts were incubated with an in vitro synthesized 630-nt 32P-labeled amastin 3'-UTR RNA.

Fig. 6 shows an audiograph of a native, low cross-linked acrylamide gel. In lane 1, the full-length amastin 3'-UTR (called A3) was incubated with extracts from amastigotes. A portion of its migration is retarded compared with that of the RNA that was not incubated with protein (lane 6). In lanes 2–5, varying amounts of amastigote protein were added to the RNA probe; however, in these four lanes heparin and RNase were also added. Heparin serves to compete off nonspecific RNA-protein interactions and RNase digests away any RNA fragment that is not protected by a bound protein. As can be seen, one large fragment is consistently protected, and as more amastigote protein is added, this fragment becomes increasingly abundant. In a similar experiment, we also saw a band shift when epimastigote extract was added. However, unlike with the amastigote protein extract, the entire band shift was caused by nonspecific RNA-protein interactions, since it was competed off with the addition of heparin and completely digested away with RNase (data not shown).

To confirm the binding seen in the non-denaturing gels, as well as to better characterize the proteins protecting the RNA, we performed UV-cross-linking followed by separation on denaturing gels. Similar to the previous experiments, the RNA probes were added to protein extracts from either amastigotes or epimastigotes. The reaction products were cross-linked with UV radiation, digested with RNase T1, separated on a SDS-PAGE gel, and detected by autoradiography. As can be seen in Fig. 7A, lane 7, 30- and 36-kDa proteins containing bound radioactivity were present in the reaction mixture to which the amastin extract had been added but were not present in the reaction to which the epimastigote extract had been added (lane 6). These bands in the amastigote extract were not de-
Fig. 7. Amastigote-specific amastin 3'-UTR RNA protection. A, a portion of in vitro transcribed amastin 3'-UTR RNA is protected from RNase digestion when UV-cross-linked with an amastigote protein extract. The full 630-nt in vitro transcribed, 32P-labeled, amastin 3'-UTR was incubated in the presence of an amastigote (A) or epimastigote (E) protein extract or no extract (N) and then cross-linked and subjected to RNase digestion, and the proteins were separated on an SDS acrylamide gel. Lane 1 has \( \frac{1}{500} \) of the amount of RNA probe added in lanes 4–9, and lanes 2 and 3 have \( \frac{1}{50} \). In the absence of cross-linking or RNase (lanes 1–3), the RNA probe is only slightly degraded. In the presence of RNase, the RNA probe is completely degraded (lanes 4 and 5). The addition of epimastigote protein extract and cross-linking does not inhibit RNA degradation (lane 6). The amastigote extract and cross-linking protects a portion of the RNA from degradation, and the protected RNA appears to be bound to proteins of approximately 30 and 36 kDa (lane 7). This protection is partially competed off by a 40-fold excess of nonlabeled full-length amastin 3'-UTR (lane 8) or by a 40-fold excess of \( \Delta_{\text{Amast}} \) nonlabeled 3'-UTR spanning the h4 and h6 domain (lane 9). B, RNase protection of the full 630-nt amastin 3'-UTR is specific. No bands appear in the absence of any protein extract or when an epimastigote protein extract is added (lanes 1 and 2). The addition of an amastigote protein extract and cross-linking protects the \( 32P \)-labeled RNA from complete degradation, and the protected radioactivity is within a band at 36 kDa (lanes 3–5). This protection is competed off slightly by a 500- and 100-fold excess of in vitro transcribed TCR27 3'-UTR (lanes 3 and 4) but is completely abolished by the addition of a 500- or 100-fold excess of the nonlabeled in vitro transcribed \( \Delta_{\text{Amast}} \) segment of the amastin 3'-UTR (lanes 6 and 7). C, amastigote protein extract, but not epimastigote protein extract, protect the \( \Delta_{\text{Amast}} \) region of the amastin 3'-UTR. The in vitro transcribed \( \Delta_{\text{Amast}} \) RNA was incubated in the presence of amastigote extract (lane 1), epimastigote extract (lane 2), or no protein extract (lanes 3 and 4). Similar to the full-length 3'-UTR in A and B, the \( \Delta_{\text{Amast}} \) region was protected by a protein of about 36 kDa. Lane 3 contains the \( \Delta_{\text{Amast}} \) RNA partially digested with RNase; lane 4 shows the migration pattern of the undigested RNA probe. D, the A3 full-length amastin 3'-UTR was incubated with protein extracts from epimastigotes or amastigotes, cross-linked, and digested with RNases. When resolved on a denaturing acrylamide gel, two protein species, 30 and 36 kDa, are bound to the RNA when amastigote protein is used (lane 3). Only a 30-kDa protein is bound when epimastigote protein is used but to a much lesser extent (lane 1). Nonradiolabeled amastin 3'-UTR RNA competes off the two proteins (lanes 2 and 4). No protection is seen when protein extract from the mammalian host cell is used (RA786; lane 5).

To examine the specificity with which this protein(s) binds to the amastin \( \Delta_{\text{Amast}} \) region, titrations of unlabeled specific and nonspecific RNA were performed. The nonspecific RNA was the in vitro transcribed TCR27 3'-UTR; the specific RNA was the full-length amastin 3'-UTR transcribed in the presence of unlabeled UTP. The image shown in Fig. 7B, lane 1, confirms the integrity of the radiolabeled full-length amastin 3'-UTR RNA probe. The full-length RNA migrates only slightly into the gel. Lane 2 confirms the absence of protected RNA when the epimastigote protein extract is used. Lane 5 of Fig. 7B was performed under the same conditions as lane 7 in Fig. 7A. Again a 36-kDa protein protected the RNA probe. The smaller 30-kDa protein, seen in Fig. 7A, may not be resolved from the 36-kDa protein on this gel. Lanes 3 and 4 of Fig. 7B show that 500- and 100-fold excesses of TCR27 3'-UTR RNA do slightly compete with the amastin 3'-UTR for binding to this 36-kDa band. However, this binding was fully eliminated when a 500- or 1000-fold excess of unlabeled full-length amastin 3'-UTR was incubated with the radiolabeled RNA (lanes 6 and 7). The protection was also maintained if the opposite strand of RNA was transcribed in vitro and used as a competitor instead of the TCR27 3'-UTR (data not shown).

Since the presence of excess unlabeled in vitro transcribed \( \Delta_{\text{Amast}} \) RNA prevented the 36/30-kDa proteins from binding the full-length amastin 3'-UTR (Fig. 7A, lane 9), we examined whether this fragment alone could bind the proteins when used...
as a probe rather than as a competitor RNA. In Fig. 7C, lane 1, it can be seen that radioactivity from the in vitro radiolabeled A2A6 region was indeed protected by a 36-kDa protein in the amastigote extract. No protection was seen when the epamastigote extract was used (lane 2). Since less RNase was used in this experiment than in the assays performed for Fig. 7, A and B, lane 3 of Fig. 7C shows a ladder of partial RNA degradation products rather than a diffuse smear. The radioactive signal in lane 4 confirms the integrity of the A2A6 RNA probe.

Panels A–C of Fig. 7 demonstrate that amastigotin proteins bind to the minimal positive regulatory sequence A2A6. Furthermore, they suggest that these proteins are approximately 30 and 36 kDa and that they bind with a relatively high affinity to the RNA. Fig. 7D shows an additional RNase protection experiment that was performed using the optimal binding conditions. Again a clear protection by 30- and 36-kDa proteins is observed. The protection of the 36-kDa protein is specific only to RNA exposed to amastigotin protein. Both epamastigotin and amastigotin protein extracts protect the 30-kDa protein; however, this protection is less extensive in the epamastigote lane.

**DISCUSSION**

It has been suggested that since trypanosomatids appear to lack polymerase II promoters, they control their mRNA steady state levels mainly through post-transcriptional means and that much of this control is exerted by the genes’ UTRs (3, 26). To date, however, there are very few well characterized cis motifs in trypanosomatid UTRs and even fewer documented RNA-protein interactions. The amastin/tuzin gene cluster is an ideal system for elucidating these post-transcriptional mechanisms, since the amastin mRNA is dramatically up-regulated in amastigotes to the point where it accounts for 1–2% of the total cellular mRNA (1).

We show here that the 68-fold difference in steady state amastin mRNA levels between amastigotes and epamastigotes is due to differences in its stability. The amastin mRNA is 7-fold more stable in amastigotes than in epamastigotes, and this prolonged half-life contributes, at least in part, to its increased abundance in amastigotes. Taken together with earlier translation inhibition data (2), these results suggest that in epamastigotes the amastin mRNA is degraded by a labile negative factor, whereas in amastigotes it is stabilized by a labile positive factor. A recent report from another group also indicates that amastin mRNA has a prolonged half-life in amastigotes (27).

In addition to the amastin 3′-UTR, the IR between the tandem amastin and tuzin genes also has regulatory effects (Figs. 2 and 4). The deletion of this IR gave a different pattern of expression when transfected into epamastigotes or amastigotes. In epamastigotes its deletion had no effect, whereas in amastigotes its deletion reduced luciferase expression by 5-fold (Fig. 2). One interpretation of this result is that the SL site of the downstream tuzin gene may affect the amastin mRNA differently in epamastigotes than in amastigotes, a possibility consistent with the reports on the coupling of trans-splicing and polyadenylation (9–11) and the involvement of polypyrimidine tracts within an IR to guide the site of polyadenylation (3). These amastin RNA processing steps may occur more rapidly and/or efficiently in amastigotes than in epamastigotes and be governed by sequences in the IR and UTR.

The 630-bp amastin 3′-UTR can be divided into three subsegments based on the regulatory functions of their sequences. The 5′-most and 3′-most regions of the 3′-UTR are dispensable for the up-regulation in amastigotes and subsequent differential expression of a reporter gene. In their absence, no change in expression pattern is seen. For example, when the 3′-most portions of the 3′-UTR were replaced by an oligonucleotide linker, no change in relative luciferase counts was seen in amastigotes. The central one-third of the 3′-UTR, however, had a profound effect on luciferase expression if it was deleted or mutated by the linker. The cis-element between h6 and h4 was necessary for maximal expression of the reporter gene in amastigotes. Intriguingly, this region alone, without the flanking portions of the amastin 3′-UTR, was not sufficient to confer stage-specific up-regulation. Complete restoration of amastigote up-regulation was evident only when the central domain of the amastin 3′-UTR was about 250 bp away from the stop codon, 180 bp away from the polyadenylation site, and flanked by the amastin IR (as typified by pLTCR27/A2A6(+/k/mid) in Fig. 4). This position dependence may reflect spatial constraints placed on the cis sequences by trans-acting factors, such as the cleavage and polyadenylation machinery that interact with them. Alternatively, the correct distance may need to be conserved in order to preserve a specific RNA secondary structure or to prevent exo- or endonucleolytic activity, such as the case for the 3′-UTRs of the T. brucei poly(ADP-ribose) polymerase genes (26) or the human α-globin and erythropoietin genes (26, 28, 29). In either event, the sequence in the A2A6 domain of the amastin 3′-UTR contains an amastigote-specific, position- and orientation-dependent, positive regulatory element. This sequence contains no previously described RNA regulatory motifs, but it is noteworthy that it is very A/U-rich. Furthermore, RNA fold programs based on the algorithms of Zuker predict that the A2A6 region can potentially fold into a thermodynamically stable group of hairpin loops (data not shown).
shown (30, 31).

The in vitro RNase protection and cross-linking assays suggest that a 36-kDa protein(s) binds the central one-third of the amastin 3′-UTR. This protein or protein complex is either more abundant in amastigotes or binds with a higher affinity in this life cycle stage of the parasite. The interaction is relatively strong and sequence-specific, since even a 500-fold excess of unrelated RNA did not completely compete off the complex. It remains to be determined if this binding protein is directly responsible for the increased steady state level of amastin mRNA in amastigotes. However, our competition and minimal binding domain analysis strongly suggest that the protein binding site is localized to the same 3′-UTR region that is crucial for maximal reporter gene expression. Therefore, it is possible that the trans-acting factors act in concert with the cis-elements to aid in the processing or stability of amastin mRNA in amastigotes.

Based on these data and earlier studies (1, 2), we propose a model for the post-transcriptional regulation of the amastintuzin gene cluster (Fig. 8). The previous nuclear run-on data indicated that the amastin-tuzin gene cluster is polycistronically transcribed to generate equal levels of precursor mRNA in amastigotes and epimastigotes (1, 2). This pre-mRNA is then processed into discrete mRNAs through the trans-splicing of a 39-nt SL sequence onto the downstream amastin pre-mRNA and 3′-polyadenylation of the upstream tuzin pre-mRNA. In epimastigotes, the amastin mRNA is quickly degraded, whereas in amastigotes its half-life is 7-fold greater. Our findings indicated that this prolonged half-life, as well as more efficient processing, is conferred by the 3′-UTR sequences in the h6–h4 domain. These sequences within the central one-third of the amastin gene’s 3′-UTR may serve to stabilize the amastin transcript when expressed in amastigotes, potentially via the binding of a labile 36-kDa protein(s). This cis-element must be positioned in the correct orientation as well as approximatively 250 nt from the stop codon and 180 nt from the polyadenylation site for it to confer its positive regulatory properties. One interpretation for this position and orientation dependence of the 3′-UTR domain and the existence of the 36-kDa binding protein is that a cis-trans interaction guides the cleavage and polyadenylation machinery and is most effective if the amastin IR is present. This scenario, as depicted in Fig. 8, is reminiscent of the cis-elements that guide the cleavage and polyadenylation system factors of higher eukaryotes (34), but further work will be necessary to determine if this amastin cis-trans interaction is truly akin to the cleavage and polyadenylation system factor system.

Post-transcriptional regulatory elements within 3′-UTRs have been found in a broad range of the phylogenetic spectrum, and their functions have been implicated in RNA stability, translation initiation, and endo- and exonuclease resistance (32, 33). Some trypanosomatid 3′-UTR sequences (e.g. those in the T. brucei poly(ADP-ribose) polymerase genes and the Leishmania gp63 genes) have been assigned both positive and negative life cycle-specific regulatory roles (13, 14). However, to date, the mechanisms responsible for the regulation have remained elusive. The results described here provide insight into the molecular mechanisms that regulate amastin mRNA levels. The restraint on the physical location of the cis-sequence, coupled with the presence of an RNA-protein interaction, suggests that this interaction actively participates in the amastin mRNA up-regulation rather than providing passive resistance to endo- or exonuclease degradation.

REFERENCES
1. Teixeira, S. M. R., Kirchhoff, L. V., and Donelson, J. E. (1995) J. Biol. Chem. 270, 22356–22359
2. Teixeira, S. M., Russell, D. G., Kirchhoff, L. V., and Donelson, J. E. (1994) J. Biol. Chem. 269, 20509–20516
3. Vanhamme, L., and Pays, E. (1995) Microbiol. Rev. 59, 223–240
4. Aly, R., Argaman, M., Halman, S., and Shapiro, M. (1994) Nucleic Acids Res. 22, 2922–2929
5. Ramamooorthy, R., Donelson, J. E., Paetz, K. E., Maybodi, M., Roberts, S. C., and Wilson, M. E. (1992) J. Biol. Chem. 267, 1888–1895
6. Hines, J. C., and Ray, D. S. (1997) Mol. Biochem. Parasitol. 89, 41–49
7. Graham, S. V. (1995) Parasitol. Today 11, 217–223
8. Hug, M., Hotz, H. R., Hartmann, C., and Clayton, C. (1994) Mol. Cell. Biol. 14, 7428–7435
9. Vassella, E., Braun, R., and Roditi, I. (1994) Nucleic Acids Res. 22, 1359–1364
10. Ullu, E., Matthews, K. R., and Tschudi, C. (1993) Mol. Cell. Biol. 13, 720–725
11. LeBowitz, J. H., Smith, H. Q., Rusche, L., and Beverley, S. M. (1993) Genes Dev. 7, 996–1007
12. Hein, A., Vassella, E., Braun, R., and Roditi, I. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 370–374
13. Hotz, H. R., Lorenz, P., Fischer, R., Krieger, S., and Clayton, C. (1995) Mol. Biochem. Parasitol. 75, 1–14
14. Ramamooorthy, R., Swihart, K. G., McCoy, J. J., Wilson, M. E., and Donelson, J. E. (1995) J. Biol. Chem. 270, 12133–12139
15. Ramamooorthy, R., Donelson, J. E., and Wilson, M. E. (1996) Mol. Biochem. Parasitol. 77, 65–76
16. Ross, J. (1995) Microbiol. Rev. 59, 423–450
17. Jacobson, A., and Peltz, S. W. (1996) Annu. Rev. Biochem. 65, 693–739
18. Higgins, C. F., Peltz, S. W., and Jacobson, A. (1992) Curr. Opin. Genet. Dev. 2, 739–747
19. Pham, V. P., Rothman, P. B., and Gottesdiener, K. M. (1997) Mol. Biochem. Parasitol. 89, 11–23
20. Pizzi, T. (1957) Ph.D. thesis, Universidad de Chile, Santiago, Chile
21. Kirchhoff, L. V., Hieny, S., Shiver, G. M., Snary, D., and Sher, A. (1984) J. Immunol. 133, 2731–2735
22. Williams, R. D., Elliot, A. Y., Stein, N., and Fraley, E. E. (1976) In Vitro 12, 623–627
23. Otsu, K., Donelson, J. E., and Kirchhoff, L. V. (1995) Exp. Parasitol. 81, 529–535
24. Otsu, K., Donelson, J. E., and Kirchhoff, L. V. (1995) Mol. Biochem. Parasitol. 75, 317–330
25. Teixeira, S. M., Otsu, K., Hill, K. L., Kirchhoff, L. V., and Donelson, J. E. (1999) Mol. Biochem. Parasitol. 105, 265–270
26. Hotz, H. R., Biebinge, S., Flaspohler, J., and Clayton, C. (1998) Mol. Biochem. Parasitol. 91, 131–143
27. Abuin, G., Freitas-Junior, L. H. G., Colli, W., Alves, M. J. M., and Schenkman, S. (1999) J. Biol. Chem. 274, 13941–13947
28. McGary, E. C., Rondon, I. J., and Beckman, B. S. (1997) J. Biol. Chem. 272, 8628–8634
29. Holck, M., and Liehhaber, S. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2410–2414
30. Zuker, M. (1994) Methods Mol. Biol. 25, 267–294
31. Zuker, M., and Stiegler, P. (1981) Nucleic Acids Res. 9, 133–148
32. Graham, S. V., and Wilson, M. E. (1992) Curr. Opin. Cell. Biol. 4, 979–983
33. Peltz, S. W., Brewer, G., Bernstein, P., Hart, P. A., and Ross, J. (1991) Crit. Rev. Eukaryotic Gene Expression 1, 99–126
34. Gravelle, B. R., Fleming, E. S., and Gilman, M. G. (1996) Mol. Cell. Biol. 16, 4942–4951
35. Teixeira, S. M. R., Kirchhoff, L. V., and Donelson, J. E. (1999) Exp. Parasitol. 93, 143–151
Amastin mRNA Abundance in *Trypanosoma cruzi* Is Controlled by a 3'-Untranslated Region Position-dependent *cis*-Element and an Untranslated Region-binding Protein

Bridget C. Coughlin, Santuza M. R. Teixeira, Louis V. Kirchhoff and John E. Donelson

*J. Biol. Chem.* 2000, 275:12051-12060.
doi: 10.1074/jbc.275.16.12051

Access the most updated version of this article at [http://www.jbc.org/content/275/16/12051](http://www.jbc.org/content/275/16/12051)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/275/16/12051.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 34 references, 15 of which can be accessed free at [http://www.jbc.org/content/275/16/12051.full.html#ref-list-1](http://www.jbc.org/content/275/16/12051.full.html#ref-list-1)