Developmental Regulation of Heat Shock Protein 83 in Leishmania

3' PROCESSING AND mRNA STABILITY CONTROL TRANSCRIPT ABUNDANCE, AND TRANSLATION IS DIRECTED BY A DETERMINANT IN THE 3'-UNTRANSLATED REGION

Received for publication, August 27, 2001
Published, JBC Papers in Press, October 11, 2001, DOI 10.1074/jbc.M108271200

Alon Zilka‡, Srinivas Garlapati‖, Edit Dahan, Victoria Yaolsky, and Michal Shapira§
From the Department of Life Sciences, Ben Gurion University of the Negev, Beer-Sheva 84105, Israel

Unique and unusual features characterize the genomes of trypanosomatids that comprise an ancient group of eukaryotes. Genes transcribed by RNA polymerase II are found in polycistronic transcription units, and gene clusters repeated in tandem frequently encode abundant proteins. There is no evidence for transcriptional activation of developmentally regulated genes, and mRNA abundance is determined exclusively by post-transcriptional mechanisms. These include differential RNA processing (1) and control of mRNA decay (2–4). Polycistronic transcripts mature by trans-splicing that adds a short capped leader (39-mer) to the 5'-end of mRNAs and by 3' processing that includes cleavage and polyadenylation. Trans-splicing and polyadenylation are coupled mechanistically in trypanosomatids and share regulatory signals that consist of polyuridylic tracks and potential AG splice sites (5, 6). The 3'-untranslated sequences lack the consensus eukaryote AUAAAA signal for cleavage and polyadenylation, and no other consensus element of that nature has been identified.

Leishmania parasites exist in the alimentary canal of female sand flies as flagellated promastigotes. They develop into virulent metacyclics that are uniquely adapted for transmission by the fly into a mammalian host where they differentiate into amastigotes, an obligate intracellular life form that resides within macrophages. Developmental gene expression is induced by environmental changes that are inflicted by the switch of hosts, including alterations in temperature (26 to 33–37 °C) and extracellular pH (7 to 5.5) (4, 7). HSP701 and HSP83 are expressed constitutively throughout the life cycle of Leishmania. However, despite the absence of transcriptional activation (2), the abundance of these transcripts increased 3–4-fold in mammalian-like temperatures because of an increase in their stability. Moreover, translation of heat shock transcripts increases by at least 10-fold upon temperature elevation (8). Above a certain threshold of temperature which varies among different Leishmania species, synthesis of cellular proteins ceases (7).

Expression of heat shock genes in eukaryotes is regulated at a variety of levels that include transcription (9), RNA processing (10) followed by export to the cytoplasm, RNA stability, and translation. In view of the conserved and universal nature of the stress response it serves as an ideal system for studying the unusual features of molecular mechanisms used by trypanosomatids and for comparing them with other eukaryotes. HSP70 transcripts in Drosophila and in yeast are degraded rapidly during recovery from heat shock at 26 °C (11, 12), with deadenylation initiating this process (13). Translation of the human and Drosophila HSP70 increases dramatically during heat shock, and it is the 5'-UTR that confers this pattern of regulation in Drosophila (14, 15). Little is known about the molecular components that control mRNA stability and its accumulation in trypanosomatids, although recent reports indicate the involvement of the 3'-UTR in the regulation of HSP70 in Leishmania (16) and in Trypanosoma brucei (17). Here we show for HSP83 in Leishmania that regulated RNA processing combined with differential decay plays a key role in determining mRNA abundance at elevated temperatures. Furthermore, the

* This work was supported by German-Israel Binational Fund Grant 1-350-062/94 and by Israel Science Foundation Grant 215/98. 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.

† These authors contributed equally to this work.

‡ To whom correspondence should be addressed: Dept. of Life Sciences, Ben Gurion University of the Negev, P. O. Box 653, Beer-Sheva 84105, Israel. Tel.: 972-8-647-2663; Fax: 972-8-647-2992; E-mail: shapiram@bgumail.bgu.ac.il.

§ The abbreviations used are: HSP70 and HSP83, heat shock proteins of 70 and 83 kDa, respectively; UTR, untranslated region; IR, intergenic region; CAT, chloramphenicol acetyltransferase; RACE, rapid amplification of cDNA ends; LC, last copy.
downstream intergenic region (IR) of HSP83 confers this pattern of regulation, and the 3′-UTR of HSP83 contains an element that induces preferential translation during heat shock.

EXPERIMENTAL PROCEDURES

Organisms—Leishmania amazonensis isolate MHOM/BR/77/LTB0016 was cultured in Schneider’s medium supplemented with 10% fetal calf serum, 4 mM l-glutamine, and 25 μg/ml gentamycin. Parasites were also grown in RPMI supplemented with 10% fetal calf serum, 4 mM l-glutamine, 25 μg/ml gentamycin, 0.0001% biotin, 0.0005% hemin, 0.002 μg/ml biotin, 40 mM HEPEs, and 0.1 mM adenosine.

Plasmid Construction—The IRs within the genomic HSP83 cluster extend from the termination codon of one gene to the first translated ATG of the following gene. This fragment contained the complete IRs at both ends. A series of constructs with deletions was created by digestion of the 2-kb BglII-SalI genomic fragment (pK58858) that encodes the HSP83 IR that provided the signals for trans-splicing of the CAT primary transcript. An HSP83 IR was then ligated downstream to the CAT gene providing the signals for 3′ cleavage and polyadenylation, resulting in plasmid pHC (formerly denoted pIC1 (2)). This gave a CAT coding gene that was flanked by HSP83 IRs at both ends. A series of constructs with deletions spanning the 3′-UTR was created by digestion of the 2-kb BglII-SalI genomic fragment (pK58858) that encodes the HSP83 IR that provided the signals for 3′ processing. The following sites were used to introduce the deletions described: Smal (201), NruI (472), SpfI (59, 655) and NcoI (873). Partial digests were performed for SpfI. The modified IRs were cloned downstream of the CAT gene in plasmid pHC, and the complete fused gene (HCH3 deletion) was cloned as a Bell-BamHI insert into the BamHI site of pH (5), an orientation opposite that of the Neo′ gene. This ensured that only the HSP83 signals would direct processing of the CAT primary transcript. A parallel set of deletions was constructed by a similar cloning approach except that the 3′-UTR was derived from the α-tubulin gene. The modified HSP83 IR was cloned downstream of the CAT gene in pALT1 (19), resulting in the pTCH-based deletion plasmids.

Transfections—Plasmid DNA was electroporated into L. amazonensis parasitites as described (19) except that a double electrical pulse of 5.5 kV/cm at 25 microfarads in a Bio-Rad Gene Pulser apparatus was applied. Stably transfected lines were selected in the presence of 60 μg/ml Geneticin (G418, Sigma). Neomycin-resistant parasites appeared 10–14 days after transfection and were grown in the presence of 200 μg/ml Geneticin.

Isolation of RNA and Northern Analysis—Total RNA was extracted from cells incubated at 26 °C and at different time points after their transfer to 33 °C with the TRI reagent (Scientific Research Laboratories). To measure the T1/2 of specific mRNAs, 10 000–100 000 parasites in 4 ml were incubated with 25 μg/ml actinomycin D and not with the corresponding 5′ probe. The intensity of hybridization was determined by scanning the autoradiograms. The transcript was more stable at 33 °C than 26 °C. The temperature was determined. A 33/26 ratio of T1/2 was corrected by nonlinear regression, and the 33/26 ratio between the T1/2 at each corresponding temperature was determined. A 33/26 ratio of T1/2 was corrected by nonlinear regression, and the 33/26 ratio between the T1/2 at each corresponding temperature was determined.

Metabolic Labeling—Parasites were grown in RPMI supplemented with a cell density of 5 × 10⁷/ml. Cells (1 ml) were preincubated at 26, 33, and 37 °C for 1 h and then labeled with 20 μCi of [35S]methionine/cysteine protein labeling mix (1,175 Ci/mmol) for 30 min at the corresponding temperatures. After labeling, the cells were harvested at 4 °C, washed twice with cold phosphate-buffered saline, and lysed in SDS-polyacrylamide gel sample buffer. Incorporation of the 35S-labeled amino acids was measured by precipitation with trichloroacetic acid. Samples of proteins containing the same amount of incorporated radiolabel corresponded to a similar number of cells and were separated over 15% SDS-polyacrylamide gels. The gels were dried and processed further for fluorography.

RESULTS

The HSP83 Gene Cluster Contains 18 Gene Copies—to evaluate the size of the HSP83 genomic cluster, L. amazonensis chromosome blocks were digested with an enzyme that does not cut within the genomic cluster but cleaves at sites located in the immediate flanking sequences. NcoI sites are found 1.2 and 0.8 kb upstream and downstream from the first and last gene copies, respectively. Southern analysis of L. amazonensis pulse field gel electrophoresis blots revealed a single 67-kb fragment that hybridized with the HSP83 probe (Fig. 1), confirming the presence of a single HSP83 genomic cluster that mapped to chromosome 28 of L. major (23). Based on the size of the repeat unit (3,580 bp (20)), the 67-kb fragment contains 18 tandem repeats of the HSP83 gene.

The HSP83 3′-UTR Confers RNA Stability at Elevated Temperatures—The temperature-dependent abundance of the

---

Fig. 1. Size estimation of the HSP83 genomic cluster by pulse field gel electrophoresis. L. amazonensis cells were embedded in agarose blocks, digested overnight with NcoI that cuts only in the flanks of the cluster, and separated by pulse field gel electrophoresis on 1% agarose gels in 0.5 × TBE. Nondigested (N.D.) blocks were separated in parallel. Running conditions included a gradient of pulses ranging from 1 to 6 min for 11 h at 6 V/cm. Gel blots were hybridized with the HSP83 probe. A mixed digest of a DNA was used as size markers.

---

2 C. L. Jaffe, unpublished data.
The 3'-IR of HSP83 confers temperature-dependent regulation on the CAT reporter mRNA. A scheme of the HSP83 genomic cluster is shown at the top, including the last gene copy and its flanking sequences (vertical bars). Cells were transfected with plasmids that contained the CAT coding gene cloned between the HSP83 5'- and 3'-IRs (white rectangles, pHCH), an α-tubulin 5'-IR (gray rectangles) combined with an HSP83 3'-IR (pTCH) and an HSP83 5'-IR and an α-tubulin 3'-IR (pHCT). DNA was extracted from cell lines grown at 26 °C and after incubation (4 h) at 33 °C. The black arrows under the DNA constructs show the RNA generated and its boundaries. Parallel cultures were incubated with actinomycin D at 26 or at 33 °C, and aliquots were removed after 1, 2, 4, and 6 h for RNA extraction. Northern blots of 10-μg RNA samples were hybridized with CAT coding gene. The same blots were stripped and rehybridized with probes that corresponded to the HSP83 coding region and rRNA. The values of the hybridization intensity were measured in a PhosphorImager and normalized according to the rRNA. Accumulation of the mRNA at 33 °C relative to 26 °C is shown as histograms. The mRNA decay curves at 26 °C (black lines, ○) and 33 °C (gray lines, ×) were obtained by nonlinear regression. The T1/2 values at 33 and 26 °C were calculated, and the ratio between them is shown (right of the decay curves).

To show that the HSP83 IRs could confer a temperature-dependent pattern of regulation, the CAT gene was cloned between two HSP83 IRs, resulting in pHCH. To examine the individual role of the 5'- and 3'-UTRs in control of mRNA stability, we placed the CAT reporter gene between IRs derived from either the HSP83 or α-tubulin gene clusters (24). This gave constructs in which the CAT gene was flanked by an upstream HSP83 IR and a downstream tubulin IR (pHCT) or by the upstream tubulin UTR and the downstream HSP83 UTR (pTCH). The IRs extended from the translational termination codon of one gene to the translational initiation of the downstream gene in the cluster, to maintain the 3' processing signals. Each of the fused genes was stably introduced into L. amazonensis cells via the pX transfection vector (25), and regulation of the CAT mRNA was investigated. When both the upstream and downstream IRs were derived from HSP83 (HCH), the pattern of regulation was similar to that of the endogenous gene. When the 3'-UTR was derived from HSP83 and the 5'-UTR was obtained from α-tubulin (TCH), the T1/2 increased 2.4-fold at 33 °C, and the steady-state level at 4 h was 1.8-fold higher. When the 3'-UTR was derived from α-tubulin and the 5'-UTR was obtained from HSP83 (HCT), the T1/2 of the resulting transcript and its steady-state level decreased by 2-fold at 33 °C (33/26 = 0.5; Fig. 2). The presence of the HSP83 3'-UTR was therefore required for increasing the T1/2 of HSP83 during heat shock.

Most Deletions in the 3'-UTR Destabilize RNA, but Abundance of These Unstable Transcripts Does Not Decrease—to map regions that may control the differential decay of HSP83 mRNA at different temperatures, we constructed a series of IRs with deletions that spanned the 3'-UTR and cloned them downstream of the CAT reporter gene (Fig. 3). The deletions did not interfere with sequences downstream to the 3'-UTR which contained the signals for 3' processing.

Initially we created small deletions that removed sequences 1–201, 201–473, 473–655, and 655–873 of the 3'-UTR. To examine whether the 5'-UTR was involved in some way in the regulation processes during heat shock, two parallel sets of the same deletions were generated using different 5'-IRs. In the TCH deletion plasmids the 5'-IR was obtained from α-tubulin, and in the HCH deletion plasmids the 5'-IR was obtained from HSP83.

3'-RACE was used to map the polyadenylation site of the wild type HSP83 and the fused CAT-HSP83 genes. This ensured that the 3'-UTRs retained their original site of polyadenylation and that the processing of the major CAT transcript occurred as expected 250 nucleotides upstream of the AG splice site of the successive gene. The poly(A)″ addition site of the endogenous HSP83 transcript was mapped 886 nucleotides downstream from the translation termination codon and 250 nucleotides upstream from the AG splice site of the subsequent gene copy in the cluster. The poly(A)″ sites of the CAT-HSP83 deletion transcripts also occurred as expected 250 nucleotides upstream from the successive site for trans-splicing. To examine the effect of the deletions in the 3'-UTR on mRNA stability at ambient and elevated temperatures, decay curves were obtained as described above, and steady-state levels were monitored at 4 h (Fig. 3, A–C). Except for Δ1–201, each of the deletions in the HSP83 3'-UTR abolished differential stability of the CAT transcript at elevated temperatures, when the
Fig. 3. Effect of 3′-UTR deletions on regulation of CAT mRNAs during heat shock. The CAT coding gene was fused with the HSP83 3′-UTR carrying a series of deletions. The 5′-UTR was derived either from HSP83 (white rectangles, pHCH-based deletions, panels A and C) or α-tubulin (gray rectangles, pTCH-based deletions, panel B). The black arrows under the DNA constructs show the RNA generated. The accumulation of CAT mRNA, the decay curves, T1/2 at both temperatures, and the ratio between them were obtained as described in the legend of Fig. 2. Data for the small deletions were obtained with pHCH-based (panel A) and pTCH-based (panel B) constructs. Data for the larger deletions and the LC IR were obtained with pHCH-based (panel C) constructs. The IR of the last gene copy in the HSP83 cluster (white rectangle) and its flanking region (vertical lines) were cloned from a cosmid library following a differential screen, using 3′- and 5′-probes (black bars above the last copy of the genomic cluster shown in Fig. 2). The IR of the LC was fused downstream to the CAT coding gene, resulting in pHCH/LC (panel C). In the absence of the endogenous signals for 3′ processing the resulting CAT transcript generated a short 3′-UTR with 73 nucleotides. A similar transcript was also obtained in cells transfected with pHCH\Delta 59–873.

5′-UTR was derived from HSP83 (Fig. 3A, HCH constructs). It is possible that all of the tested modifications could alter the structure of the 3′-UTR and therefore change the accessibility of regulatory determinants. If the 5′-UTR was derived from α-tubulin all of the deletions eliminated differential stability, including Δ1–201 (Fig. 3B, TCH constructs). Furthermore, the destabilizing effect of deletions in the 3′-UTR as elucidated by the 33/26 ratio of T1/2 was more evident in combination with the α-tubulin 5′-UTR. This could indicate possible interactions between the 5′- and 3′-UTRs of HSP83. In both sets of constructs, deletion 201–472 in the 3′-UTR had the most profound effect on eliminating differential stability.
We noted that although the tested deletions (200–250 nucleotides) eliminated differential stability of the CAT mRNA at elevated temperatures, the resulting transcripts accumulated to comparable or slightly higher levels at both temperatures (Fig. 3, A and B). This suggested that temperature-dependent degradation of mRNAs was not the only mechanism acting post-transcriptionally to control mRNA abundance in Leishmania.

To increase the instability of the 3'-UTR at 33°C and to examine the effect on accumulation of the CAT transcript, we introduced larger deletions into the 3'-UTR, spanning sequences A59–472, A472–873, A59–655, and ΔA59–873. These deletions led to a marked destabilization of the CAT transcripts at elevated temperatures, decreasing the 33/26 T1/2 ratio to 0.3–0.5. However, despite this dramatic reduction in mRNA stability, the CAT transcripts accumulated to comparable (0.97, pHCHA59–873) or mildly reduced (0.67, pHCHA659–472; 0.79, pHCHA472–873; 0.74, pHCHA59–655) steady-state levels at 33°C (Fig. 3C). This could suggest that the abundance of the CAT transcripts was determined not only by differential mRNA stability. If 3' cleavage and polyadenylation were more efficient at 33°C, their influence could overcome the effect of reduced mRNA stability at elevated temperatures because of the deletions in the 3'-UTR. Thus, transcript abundance most probably resulted from the combination between the different regulatory processes.

The Last HSP83 Gene Copy Contains Sequences Homologous to the 3'-UTR but Lacks the Endogenous Signals for 3' Processing.—To investigate further the effect of specific 3' processing signals on transcript abundance during heat shock we used the IR of the terminal HSP83 gene in the genomic cluster. This last gene copy and 3 kb of its nonhomologous flanking sequences were cloned from a cosmid library of L. amazonensis. This was done by a differential screen using probes derived from the 5'- and 3'-ends of the coding region (Fig. 2A, scheme at the top describing the HSP83 cluster), as described previously for cloning of the last HSP83 repeat unit (21). The terminal IR sequence is almost identical (99%) to that of an internal repeat unit (1,471 bp) until position 840, from which point the two sequences are totally divergent.

Initially it was unclear whether the last copy (LC) was expressed because a putative poly(A)polymerase track and AG sites that could serve as signals for 3' processing of the last transcript in the cluster were observed 500 bp downstream to position 840 in the terminal IR. The IR of the last gene copy and 0.5 kb of the flanking sequences were therefore fused downstream to the CAT coding gene (pHCH/LC, Fig. 3C) and stably introduced into L. amazonensis cells. The polyadenylation site of the corresponding CAT transcript was examined by 3'-RACE and quite surprisingly, was mapped 73 nucleotides after the translational termination codon. This indicated that the flanking genomic sequences located downstream from the LC failed to direct 3' processing at the accurate site, and the resulting transcript was devoid of most of its 3'-UTR. Cleavage and polyadenylation that took place 73 nucleotides downstream from the translational termination codon were most probably directed by cryptic signals. The boundaries of the CAT transcripts produced by the pHCH59–873 and pHCH/LC constructs were therefore similar, each containing a very short 3'-UTR of 73–84 nucleotides. However, despite the structural similarity between the two transcripts, their pattern of regulation varied (Fig. 3C). Both mRNAs were equally unstable at 33°C with a 33/26 ratio for T1/2 of 0.3, but they differed in their ability to maintain high steady-state levels of RNA at elevated temperatures. Although the HCH59–873 CAT transcript accumulated to a comparable steady-state level at both temperatures (33/26 ratio = 0.97 at 4 h), the abundance of the HCH/LC mRNA decreased after incubation at elevated temperatures (33/26 = 0.25). This could be explained by a reduction in the processing efficiency of 3'-ends in the HCH/LC transcript at elevated temperatures, which affected its RNA steady-state levels. Thus it appeared that the last gene copy in the HSP83 cluster lacks the endogenous signal for 3' processing and is most probably not expressed at elevated temperatures.

Processing of Heat Shock mRNA Transcripts Is More Efficient at Elevated Temperatures.—To examine the effect of temperature elevation on processing of heat shock transcripts, total RNA was isolated from cells grown at 26°C and after exposure to 33°C (4 h). The RNA was fractionated over a poly(dT)-cellulose column, and the poly(A)+ and poly(A)- fractions were blotted and hybridized with HSP70, HSP83, and α-tubulin probes. As expected, the mature HSP83 transcripts (3.1 and 3.3 kb, respectively), although present at both temperatures were more abundant at 33°C (Fig. 4). Hybridization with the poly(A)+ fractions revealed the presence of larger faint bands only in RNA obtained from 26°C. These bands were not observed in samples extracted from 33°C and could correspond to unprocessed transcripts, possibly dimers and trimers. Their abundance at the lower temperature suggested that processing of heat shock transcripts under these conditions was less efficient. Processing of the tubulin transcripts did not seem to vary with temperature alteration.

Sequences within the 201–472 of the 3'-UTR Are Required for Preferential Translation of Heat Shock Transcripts.—Heat shock transcripts in Leishmania are translated preferentially at temperatures typical of the mammalian host (8). Previously we showed for L. amazonensis that the increase in translation of HSP70 and HSP83 was observed at 33°C, whereas translation of tubulin and other cellular non-heat shock proteins ceased at 37°C (7). We observed a similar pattern of regulation for the CAT mRNA flanked by HSP83 3'- and 5'-UTRs (Fig. 5 and Ref. 18), therefore determined the location of the regulatory elements. This was done by incorporation of radioactive amino acids into nascently synthesized proteins for 30 min in cells grown at 26°C and after exposure to 33 and 37°C for 1 h. We show for Leishmania that the 3'-UTR derived from the HSP83 gene regulates the efficiency of translation at different temperatures. Translation of CAT increased at 33°C if it was fused to the HSP83 3'-UTR (pTCH). When the HSP83 3'-UTR was exchanged with the α-tubulin 3'-UTR preferential trans-
sequences within the 201–472 region of the larger deletions. Removal of the proximal half (59–655, and 655–873), and cells transfected with these plasmids could not preferentially translate their CAT transcript.

**DISCUSSION**

We show that the 3′-UTR confers differential stability on the HSP83 mRNA in *Leishmania* because CAT transcripts fused to an HSP83 3′-UTR and an α-tubulin 5′-UTR followed the same pattern of temperature-dependent regulation observed for the endogenous HSP83 gene. These CAT transcripts were more stable and accumulated to a higher level at 33 °C than at 26 °C. Reciprocal constructs that contained an α-tubulin 3′-UTR and an HSP83 5′-UTR were degraded faster at 33 °C and were less abundant at that temperature compared with 26 °C. Decay curves at different temperatures revealed that the HSP83 5′-UTR had a limited synergistic effect on mRNA stability during heat shock when combined with the HSP83 3′-UTR. The HSP83 upstream IR may differentially accelerate trans-splicing of the 5′-ends, as also described for the phosphoglycerate kinase genes in *T. brucei* (26).

Analysis of the 3′-UTR was performed by introducing deletions of 200–250 nucleotides downstream from the CAT gene in constructs that contained a 5′-UTR derived from α-tubulin. This showed that there was no specific region involved in temperature-dependent control of HSP83 mRNA stability. All deletions examined generated mRNA molecules that were no longer stable at 33 °C, although removal of sequences 201–472 displayed the strongest effect. This could indicate that the 3′-UTR created secondary structures that were involved in regulation of mRNA stability and were disrupted by each of the deletions. Sequences 1–201, a region that is least conserved among *Leishmania* species (27), were dispensable only if the 5′-UTR was derived from HSP83. This observation could indicate possible interactions between the 3′- and 5′-UTRs, despite the inability of the 5′-UTR by itself to confer preferential mRNA stability at elevated temperatures. The larger deletions that eliminated half (either the proximal or the distal) or larger parts of the 3′-UTR had a more profound effect on differential stability of mRNA, and the T1/2 of the resulting CAT transcripts decreased by 2–3-fold upon heat shock.

Although most of the deletions that removed 200–250 nucleotides from the 3′-UTR abolished the increased mRNA stability at elevated temperatures, they did not prevent the accumulation of the CAT-HSP83 transcript to higher levels. Only the more dramatic decrease in T1/2 at 33 °C which was observed with the larger deletions generated CAT transcripts that no longer accumulated to higher levels at 33 °C. We therefore examined whether a more efficient processing of HSP83 mRNAs at elevated temperatures could compensate for the reduction in stability. This was done by analysis of two mRNA molecules that had similar boundaries but varied in their 3′ processing signals. One was obtained from an internal HSP83 IR and the other from the last gene copy in this cluster. We found that processing signals of the internal HSP83 IR were effective and functional at elevated temperatures, but they were absent from the last gene copy. Although putative signals for 3′ processing were observed within 0.5 kb of the sequences that flanked the terminal gene copy, they proved to be nonfunctional. Thus, CAT transcripts in cells transfected with either plasmid were polyadenylated very close to the translational termination site. Although both transcripts were devoid of a 3′-UTR and had similar stability values (33/26 ratio of 0.3 for
In trypanosomes, a severe heat shock (42 °C) treated with a milder stress that prevents this inhibition (10). HSP70 genes in Drosophila are intronless, enabling their maturation during extreme conditions. However, a severe temperature treatment in Leishmania, suggested that the nonstructured AT-rich 5'-UTR is removed but not when the proximal half of the 3'-UTR (59–125) was replaced with an α-tubulin 3'-UTR abolished preferential translation even if the 5'-UTR was derived from HSP83.

We identified a regulatory element located between positions 201 and 472 in the 3'-UTR of HSP83 which enhanced translation in Leishmania during heat shock. Translation increased at 33 °C even if the distal half of the 3'-UTR (472–873) was removed but not when the proximal half of the 3'-UTR (59–472) was deleted, in correlation with the presence or absence of this element. Moreover, preferential translation occurred even in transcripts that were less stable at 33 °C because of mutations in the 3'-UTR as long as the 201–472 region was present. This was best exemplified by the CAT mRNA in pCH4Δ72–873 cells that translated more efficiently at elevated temperatures, despite being less stable. Thus, translational regulation and control of mRNA stability of HSP83 in Leishmania are independent processes. The dissociation between stability and translation efficiency of RNA is usually not common among eukaryotes; however, it has been observed in some cases. For example, an element within the 3'-UTR of c-fos links the translation and intracellular localization of this transcript but has no bearing on its stability (36).

Elements that control translational regulation in eukaryotes are generally found in the 5'-UTR. However, there are several examples of transcript-specific factors that affect translational regulation via elements in the 3'-UTR. The mouse quaking proteins (QKI) involved in embryogenesis and myelination and a closely related protein in Caenorhabditis elegans (GLD-1) which is necessary for germ line development are members of the STAR family of RNA-binding proteins. Both proteins were found to serve as translational repressors that act through regulatory elements called TGEs (for tro-2 and GLI elements), present in the 3'-UTR of their target genes (37, 38). During axis formation in Drosophila, translation of hunchback is repressed by the gene products of nanos and pumilio via binding to defined elements in its 3'-UTR (39, 40). An element within the 3'-UTR of the lipoygenase transcript is responsible for its silencing during erythroid differentiation (41). In T. brucei, a 26-mer sequence in the 3'-UTR of the major surface antigen of procyclic trypanosomes controls the developmentally regulated abundance of the procyclic acidic repetitive antigen (PARP) transcript and the protein it encodes (42, 43). Although translational regulatory elements are occasionally found within 3'-UTRs, the 5'-UTR of heat shock genes in eukaryotes accounts for their traditionally conserved translational regulation. However, the evidence presented here suggests that a 3'-UTR determinant is responsible for preferential translation of HSP83 in Leishmania, illustrating another aspect of unique and unusual regulatory pathways employed by trypanosomatids.

Acknowledgments—We are grateful to D. Smith from Imperial College, London, for the L. amazonensis cosmid library, to S. Beverley from Washington University, St. Louis, for the pX vector, and to D. Wirth from Harvard School of Public Health for the pALT1 DNA constructs.

REFERENCES

1. Muhich, M. L., and Boothroyd, J. C. (1989) J. Biol. Chem. 264, 7167–7110
2. Argaman, M., Aly, R., and Shapira, M. (1994) Mol. Biochem. Parasitol. 64, 95–110
3.Brandau, S., Dresel, A., and Clos, J. (1995) Biochem. J. 310, 225–232
4. Charest, H., Zhang, W.-W., and Matlashewski, G. (1996) J. Biol. Chem. 271, 17081–17090
5. LeBowitz, J. H., Smith, H., and Beverley, S. M. (1993) Genes Dev. 7, 996–1007
6. Matthews, K. R., Tschudi, C., and Ullu, E. (1994) Genes Dev. 8, 491–501
7. Garlapati, S., Dahan, E., and Shapira, M. (1999) Mol. Biochem. Parasitol. 106, 99–101
8. Shapira, M., McEwen, J. G., and Jaffe, C. L. (1988) EMBO J. 7, 2895–2901
9. Morimoto, R. (1998) Genes Dev. 12, 3788–3796
10. Lee, M.-S. (1998) Mol. Cell. Biol. 18, 1062–1068
11. Theodorakis, N. G., and Morimoto, R. I. (1987) Mol. Cell. Biol. 7, 4357–4368
12. Petersen, R., and Lindquist, S. (1988) Gene (Amst.) 72, 161–168
13. Delavalle, R. P., Petersen, R., and Lindquist, S. (1994) Mol. Cell. Biol. 14, 3646–3659
14. Lindquist, S. (1981) Nature 293, 311–314
15. Vivinus, S., Baulande, S., van Zanten, M., Campbell, F., Topley, P., Ellis, J. H., Dessen, P., and Coste, H. (2001) Eur. J. Biochem. 268, 1908–1917
16. Quijada, L., Soto, M., Alonso, C., and Requena, J. M. (2000) Mol. Biochem. Parasitol. 110, 79–91
17. Lee, M.-S. (1996) Nucleic Acids Res. 24, 4025–4033
18. Aly, R., Argaman, M., and Shapira, M. (1994) Nucleic Acids Res. 22, 2922–2929
19. Leban, A., and Wirth, D. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9119–9123
20. Shapira, M., and Pedraza, G. (1999) Mol. Biochem. Parasitol. 42, 247–256
21. Argaman, M., Pinelli, E., and Shapira, M. (1993) Gene (Amst.) 127, 155–167
22. Pinelli, E., and Shapira, M. (1999) Eur. J. Biochem. 264, 685–691
23. Samaras, N., and Sipitaki, T. W. (1987) Mol. Biochem. Parasitol. 25, 279–291
24. Landfeur, S., McMahon-Pratt, D., and Wirth, D. (1983) Mol. Cell. Biol. 3, 1070–1076
25. LeBowitz, J. H., Coburn, C. M., McMahon-Pratt, D., and Beverley, S. M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9746–9750
26. Kapatos, N., and Bellottato, V. (1993) Nucleic Acids Res. 21, 4067–4072
27. Aly, R., Argaman, M., and Shapira, M. (1995) Exp. Parasitol. 80, 159–162
28. Yost, H. J., and Lindquist, S. (1986) Cell 45, 185–193
29. Bond, U. (1988) EMBO J. 7, 3509–3518
30. Bracken, A. P., and Bond, U. (1990) RNA 12, 1586–1596
31. Muhich, M. L., and Boothroyd, J. C. (1988) Mol. Cell. Biol. 8, 3837–3846
32. Muhich, M. L., Hsu, M. P., and Boothroyd, J. C. (1989) Gene (Amst.) 82, 169–175
33. Klenzien, R., Huerten, D., and Gehring, W. J. (1985) EMBO J. 4, 2053–2060
34. McGarry, T. J., and Lindquist, S. (1985) Cell 42, 983–991
35. Hans, M. A., and Duncan, R. D. F. (1994) J. Biol. Chem. 269, 10913–10922
36. Dalgleish, G., Veyrune, J.-N., Blanchard, J.-M., and Hesketh, J. (2001) J. Biol. Chem. 276, 10913–10922
Developmental Regulation of HSP83 in Leishmania

37. Jan, E., Motny, C. K., Graves, L. E., and Goodwin, E. B. (1999) EMBO J. 18, 255–269
38. Saccomanno, L., Loushin, C., Jan, E., Punkay, E., Artzt, K., and Goodwin, E. B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12605–12610
39. Murata, Y., and Wharton, R. P. (1995) Cell 80, 747–756
40. Wharton, R. P., Sonoda, J., Lee, T., Patterson, M., and Murata, Y. (1998) Mol. Cell 6, 863–872
41. Ostareck, D. H., Ostareck-Lederer, A., Wilm, M., Thiele, B. J., Mann, M., and Hentze, M. (1997) Cell 89, 597–606
42. Hotz, H.-R., Biebinger, S., Flaspohler, J., and Clayton, C. E. (1998) Mol. Biochem. Parasitol. 91, 131–143
43. Furger, A., Schürch, N., Kurath, U., and Roditi, I. (1997) Mol. Cell. Biol. 17, 4372–4380