Lights and shadows on gene organization and regulation of gene expression in *Leishmania*

Jose M. Requena

Centro de Biologia Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain

TABLE OF CONTENTS

1. ABSTRACT

Regulation of gene expression is one of the most intriguing aspects of *Leishmania* biology. This review deals with current knowledge concerning gene organization and regulation of gene expression in this protozoan parasite, which cause serious illness and death in humans living in tropical and subtropical regions. Post-transcriptional regulation is especially important for *Leishmania*, and other trypanosomatids, due to the unusual features related to transcription and mRNA maturation. In these organisms, genes are organized into polycistronic transcription units, whereby many genes are cotranscribed by RNA polymerase II from not well characterized, upstream promoters. These organisms represent an extreme in which the expression of their genome is almost exclusively controlled post-transcriptionally. Because the regulatory needs of these parasites are considerable as they undergo complex developmental transitions, post-transcriptional mechanisms that involve RNA and protein regulatory processes are of paramount importance for these protozoa. This review summarizes recent results on the post-transcriptional mechanisms in *Leishmania* that regulate protein abundance through influencing RNA splicing, nuclear-cytoplasmic mRNA stability, translation, or post-translational events such as protein stability and modification.

2. INTRODUCTION

Leishmania are protozoan parasites, aetiological agents of a spectrum of clinical diseases, known as leishmaniases, ranging from disfiguring skin lesions to life-threatening visceral infection. The parasite has a world-wide distribution and the World Health Organization (WHO) estimates that 350 million people are at risk of infection. Two million new cases (1.5 million for cutaneous forms and 500 000 for visceral leishmaniasis) are considered to occur annually, with an estimated 12 million people presently infected worldwide. Over the last 10 years, endemic regions have been spreading further and there has been a sharp increase in the number of recorded cases of the disease (1).

Leishmania are transmitted between vertebrate hosts by phlebotomine sand flies of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World). In the sand fly, and in culture medium, the parasite multiplies as extracellular forms named promastigotes (Figure 1). In the sand fly midgut, *Leishmania* promastigotes undergo a process of programmed differentiation from a relatively moninfective stage to a highly infective metacyclic stage (2). Once inoculated, the metacyclics are phagocytosed by macrophages, in which they transform into non-motile, intracellular, replicative amastigotes and become resident
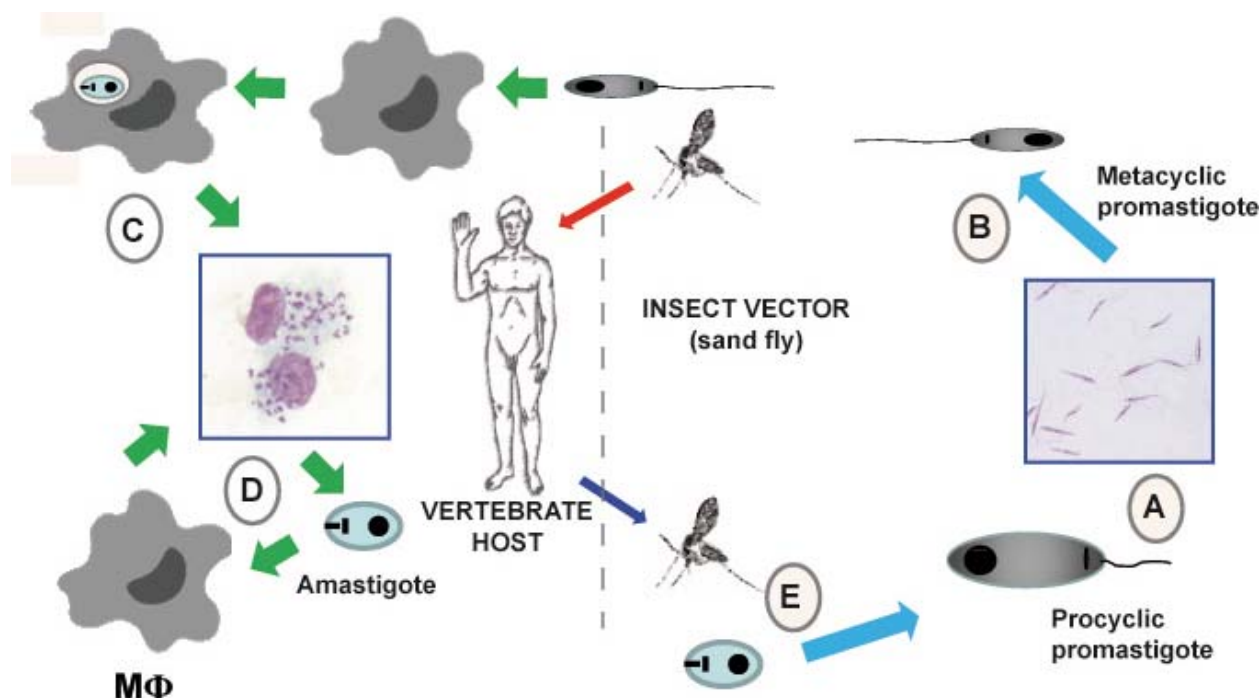
Figure 1. The life cycle of *Leishmania*. The parasites are transmitted to vertebrate hosts by short-lived phlebotomine sand flies (*Phlebotomus* spp. and *Lutzomyia* spp.). In the midgut of their vector, or in culture medium (A), they multiply as procyclic promastigote forms with an anterior flagellum. Prior to transmission to the vertebrate, they differentiate into non-replicative, infective metacyclic forms (B), which are adapted for survival in the mammalian hosts. Once inoculated, the metacyclics are phagocytosed by macrophages (M-phi), in which they transform into non-motile, intracellular, replicative amastigotes and become resident within one or more specialized parasitophorous vacuoles (C). Amastigotes divide by binary fission within macrophage phagolysosomes and eventually burst free from infected macrophages, spreading the disease within the mammalian host (D). When a sand fly bites an infected host, *Leishmania* amastigotes residing macrophages are taken up in the blood meal (D); the cycle is completed when the ingested amastigotes differentiate into the replicating procyclic promastigote stage.

within one or more specialized parasitophorous vacuoles. This new step of differentiation is a prerequisite for the intracellular survival of the parasite. The molecular basis of this transformation remains poorly understood although it is well established that several environmental factors such as pH and temperature trigger this cytodifferentiation process (3). Thus, one area of special interest is to address how the expression of genes is tightly regulated, allowing *Leishmania* to adapt rapidly to sudden environmental changes. However, despite the identification of several stage-specific genes in *Leishmania*, the molecular mechanisms governing developmental gene regulation remain not well understood.

The genus *Leishmania* belongs to the family of trypanosomatids, which also include, among others, *Trypanosoma brucei* and *Trypanosoma cruzi*, causative agents of other important infectious diseases for humans (sleeping sickness and Chagas disease, respectively). In addition, these pathogens occupy a deep-branched position on the evolutionary tree of eukaryotes (4). This divergence is reflected in the fact that trypanosomatids exhibit a number of highly original molecular and cellular biological processes, such as RNA editing of mitochondrial transcripts, systematic *trans*-splicing (consisting in the addition of a 39 nucleotide-spliced leader RNA at the 5'

end of all mRNAs), and the near absence of promoters for RNA polymerase II, which implies an absence of regulation of gene expression at the transcriptional level. One of the most extraordinary features revealed by sequencing of their genomes is the gene organization: genes are arranged into large collinear clusters present on a single strand and comparable to prokaryotic polycistronic units, except that the genes present have no common or akin function (5-7). These large directional gene clusters are separated by short sequences of a few kilobases termed coding strand switches or strand-switch regions, where the transcription initiates (8). Hence, the gene clusters are transcribed into polycistronic RNA precursors that are further processed into individual mRNAs by *trans*-splicing and polyadenylation (9). In this situation, there is no place for regulation at the transcriptional level and, in pursuing differential expression of individual genes, trypanosomatids have potentiated alternative mechanisms of gene expression, such as differential processing of polycistronic transcripts, regulation of mRNA stability and translation, post-translational modification of proteins, and regulation of protein half-life.

Regulation of gene expression in trypanosomatid parasites has been addressed in excellent reviews (10-12). Although trypanosomatids have similar gene organization

and share global mechanisms of gene expression, peculiarities exist in the different groups of trypanosomes. This review is centred mostly on the current knowledge and salient features of regulation of gene expression in *Leishmania*. A comprehensive review linking aspects of genomic organization and gene regulation in *Leishmania* was published more than ten years ago (13), and it was time to bring it up to date. In part, this gap was filled with the publication of a book chapter on regulation of gene expression in *Leishmania* (14); hence, in preparing this review, two purposes were pursued: to give a complementary view to previous reviews and including recent achievements in this fast evolving field.

3. CHROMOSOMAL ORGANIZATION AND GENE TRANSCRIPTION

Genome projects in various *Leishmania* species (5, 15) have revealed extraordinary features regarding gene organization in these parasites. Ten to hundreds of protein-coding genes are organized into large collinear clusters present on a single strand and comparable to prokaryotic polycistronic units, except that the arranged genes generally do not code for functionally related proteins. These large directional gene clusters (DGCs) are separated by short sequences of a few kilobases termed strand-switch regions (SSRs), where the transcription senses converge or diverge. This gene organization is a consequence of the unconventional mode of transcription in *Leishmania*: transcription is polycistronic and gene-specific promoters are absent or very rare in their genome. RNA polymerase II (pol II) initiates transcription within the SSRs in a strand-specific manner and proceeds along the cluster, yielding then polycistronic transcripts (8, 16). Hence, in contrast to most organisms, *Leishmania* (and other trypanosomatids) seem to have lost, or never acquired, the ability to regulate transcription initiation of individual genes (17). Although post-transcriptional regulation seems the only regulatory way for trypanosomatids, it is becoming clear that, also for many organisms, post-transcriptional control represents a regulatory network of a complexity and importance likely greater than transcriptional control (18).

In *Leishmania* and related trypanosomatids, most protein-encoding genes are transcribed in large polycistronic transcription units (PTUs) and then processed to monocistronic mRNAs by the mechanism of *trans*-splicing and polyadenylation (see below). Thus, pol II transcription starts at SSRs between two transcriptionally divergent PTUs and ends at SSRs between two transcriptionally convergent PTUs. However, the mechanism by which transcription is initiated has to be elucidated, and the promoter sequences remain undefined. In *L. major*, a recent study on chromosomal localization of several DNA-binding proteins by chromatin immunoprecipitation (ChIP) has shown that acetylated H3 histones accumulate at the origins of polycistronic transcription. Hence, global regulation of transcription initiation might be achieved by modifying the acetylation state of H3 histones located at the SSRs (19). Remarkably, immediately upstream of the acetyl-H3 peaks, accumulation of transcription factors like TBP (TATA-binding protein) and SNAP (Small Nuclear Activating Protein) were often observed (19). In *T. brucei*, using also ChIP, it has been found that the beginning and end of PTUs are characterized by the presence of distinct histone variants. Thus, H2AZ and H2BV are enriched at probable pol II transcription start sites, whereas H3V and H4V are enriched at probable transcription termination sites (20).

4. COPY NUMBER OF GENES AND REGULATION OF GENE EXPRESSION

Evidence from gene knockouts, restriction-site polymorphisms, genetic recombination and karyotypic analyses points to a diploid genomic content in *Leishmania* (13). Interchange of genetic material, even infrequent, was described in *Leishmania*; however, there is no evidence about the existence of sexual reproduction mechanisms which may explain a diploid genome (21). Therefore, another functional pressure must be responsible of maintaining a diploid content of genes. In some studies, it has been documented that gene deletion by targeted replacement of one allele results in dramatic effects on growth rate and fitness of the heterozygous mutants in comparison to the wild-type parasites. Thus, for example, deletion of one allele of the glyoxalase I gene in *L. donovani* promastigotes resulted in heterozygous mutants exhibiting reduced methylgyoxal detoxification (22). Also, mutants with one inactivated-allelic copy of the gamma-glutamylcysteine synthetase are more susceptible to oxidative stresses *in vitro* as promastigotes and show decreased survival inside activated macrophages (23). Similarly, deletion of one allele of the *L. infantum* silent information regulatory 2 (LiSIR2) gene was sufficient to dramatically affect amastigote axenic proliferation, and, furthermore, LiSIR2 single knockout (LiSIR2(+/-)) amastigotes were unable to replicate *in vitro* inside macrophages (24).These findings suggest that, at least for some genes, both alleles need to be transcriptionally active to achieve a steady-state level of transcripts adequate for normal cell growth.

Another remarkable aspect of *Leishmania* biology is the observation that, as consequence of exposure to stress conditions such as drug pressure or nutritional deprivation, the parasite can replicate large chromosomal regions in the form of extrachromosomal elements (25, 26, 27). Interestingly, a recent study has shown that *Leishmania* may experience amplification of entire chromosomes, and even the loss of one chromosome, under drug pressure (28). This genomic plasticity seems to constitute an emergency solution for *Leishmania* survival, allowing a higher expression of particular proteins to cope with stressful conditions.

Tandemly repeated, multi-copy gene loci are frequent in the *Leishmania* genome (13, 29). Furthermore, it is likely that many repeated genes (and their copy number) are not correctly annotated in the *Leishmania* genome databases due to the difficulty in assembling tandemly repeated sequences after shot-gun sequencing. Highly expressed proteins as tubulins, heat shock proteins, proteases, glucose transporters and surface antigens, among

others, are encoded by genes present in multiple copies. Therefore, tandemly repetition of genes may be envisioned as a direct mechanism to increase the transcript abundance of highly expressed proteins. The importance of the copy numbers has been demonstrated in studies analyzing molecular karyotypes and virulence of *Leishmania* strains (27). For example, less pathogenic parasites present a lower number of rDNA genes (30), a lower copy number of genes encoding the surface antigen PSA-2 (31), or fewer genes for surface protease GP63 than more virulent strains (32).

A remarkable feature of *Leishmania* genome is the fact that a significant number of repeated genes consists of two tandemly arranged copies showing a remarkable conservation in the coding regions but significant divergence in the untranslated regions (UTRs). Examples of loci with such gene organization are those coding for acidic ribosomal proteins P2a, P2b and P0 (33, 34), methionine adenosyltransferase (35), arginine-specific transporter LdAAP3 (36), cation transporting ATPase (37), and T-protein component of the glycine cleavage complex (38). The presence of divergent 3'-UTRs, but conserved coding regions, is also observed in gene tandems composed of more than two copies (e.g., genes encoding surface protease GP63 (39), β-tubulin (40), HSP70 (41), HSP83 (42), and histone H2A (43)). For many of these genes, the steady-state levels of both types of transcripts are different and often are differentially regulated during *Leishmania* development. An illustrative example can be found in the organization of genes coding for the most abundant surface protease (MSP or GP63) of *Leishmania* (see reference 44 for a review). Briefly, the *MSP* locus contains at least 18 tandemly arranged genes encoding MSP in *L. chagasi*, which fall into three classes (*MSPL, MSPS* and *MSPC*) according to their unique 3'-UTRs. The mRNAs encoded by these genes are differentially expressed along the growth curve. Thus, *MSPL* mRNAs are expressed predominantly in logarithmic phase of promastigote growth curve, *MSPS* mRNAs are exclusively expressed in stationary phase, and the single copy *MSPC* gene is constitutively expressed throughout both growth phases. Thus, events of gene duplication and divergence in the 3'-UTR regulatory sequences can be envisaged as an evolutionary strategy followed by *Leishmania* to ensure adequate expression of a relevant gene during the different stages of the parasite life cycle, when alternative mechanisms of post-transcriptional regulation associated with specific 3'-UTR *cis*-elements might be affecting differentially the expression of each "isogenic" transcript.

5. REGULATION OF mRNA PROCESSING

mRNA maturation in trypanosomatids is linked to the polycistronic transcription and differs from the process in most eukaryotes. Polycistronic mRNAs are dissected into monocistronic mRNAs by coupled reactions of *trans*-splicing and polyadenylation (45). The process of *trans*-splicing, consisting in the addition of a common 39-nucleotide sequence (known as spliced leader -SL- or miniexon) at the 5'-end of all mRNAs, serves additionally the purpose of providing the cap structure to mRNAs (46). For a detailed description of the mechanism of *trans*-

splicing in trypanosomatids, see the comprehensive review by Liang and co-workers (9). The mechanism of *trans*-splicing shares several features with *cis*-splicing: for instance, the 3'-splice acceptor site consists of the invariant dinucleotide AG and an upstream polypyrimidine tract of variable length (47). Like *cis*-splicing, *trans*-splicing occurs via two transesterification reactions, but instead of a lariat intermediate a Y structure is formed during the first step of the reaction (9). A remarkable feature observed during the processing of polycistronic transcript is that polyadenylation and *trans*-splicing processes are coupled, i.e. the *trans*-splicing at the 5'-end of a gene precedes polyadenylation of its upstream neighbour (45). No specific sequences for polyadenylation have been identified in *Leishmania* and the selection of the site seems to be determined mainly by both the presence of a downstream polypyrimidine tract and the presence of an active splice-acceptor site located within the 200-500 nucleotides downstream (48). In this context, intergenic (or intercistronic) sequences, present in the polycistronic transcripts but absent from the mature mRNAs, may influence the rate and efficiency of maturation of the different mRNAs derived from the same transcriptional unit. Experimental evidence of a direct involvement of intercistronic sequences in gene expression has been obtained from the analysis of stage-regulated expression of *L. mexicana* CPB cysteine proteases (49). The CPB locus in *L. mexicana* is composed of 19 tandemly arranged genes, but they are expressed at variable levels in the different developmental stages. It has been demonstrated that the presence of a DNA element of 120 bp (termed the insertion sequence, InS), downstream of CPB1 and CPB2 genes, is responsible for the metacyclic-specific expression (and downregulation in the amastigote form) of these genes. Hence, it was proposed that the InS affects the maturation of CPB pre-mRNA in the amastigote stage (49).

During developmental differentiation of *L. donovani*, from promastigote to amastigote forms, different populations of medRNAs (SL precursors) are observed (50). Thus, two transcripts of different sizes are detected in amastigotes, one of approx. 86-nucleotides (nt) and a second larger transcript of approx. 170-nt, whereas only the 86-nt transcript is observed in promastigotes. The 170-nt transcript bears 15 additional nt at its 3'end and is polyadenylated in contrast to the 86-nt RNA. The poly(A)+ medRNAs represent 12-16% of the total SL transcripts in amastigotes and are synthesized from one class of the mini-exon genes (50). It is likely that both types of medRNAs have different efficiencies in *trans*-splicing, even though the functional role played by the poly(A)+ medRNAs in amastigotes has not been addressed.

Once processed, mature mRNAs need to be exported to the cytoplasm in order to be translated. In *Leishmania* and related trypanosomatids, little information is available on the shuttling of mRNAs (and the composition of ribonucleoprotein complexes) through the nuclear pores into the cytoplasm, but regulation at this stage is clearly possible (10). Once in the cytoplasm, the mRNA must associate with proteins required for
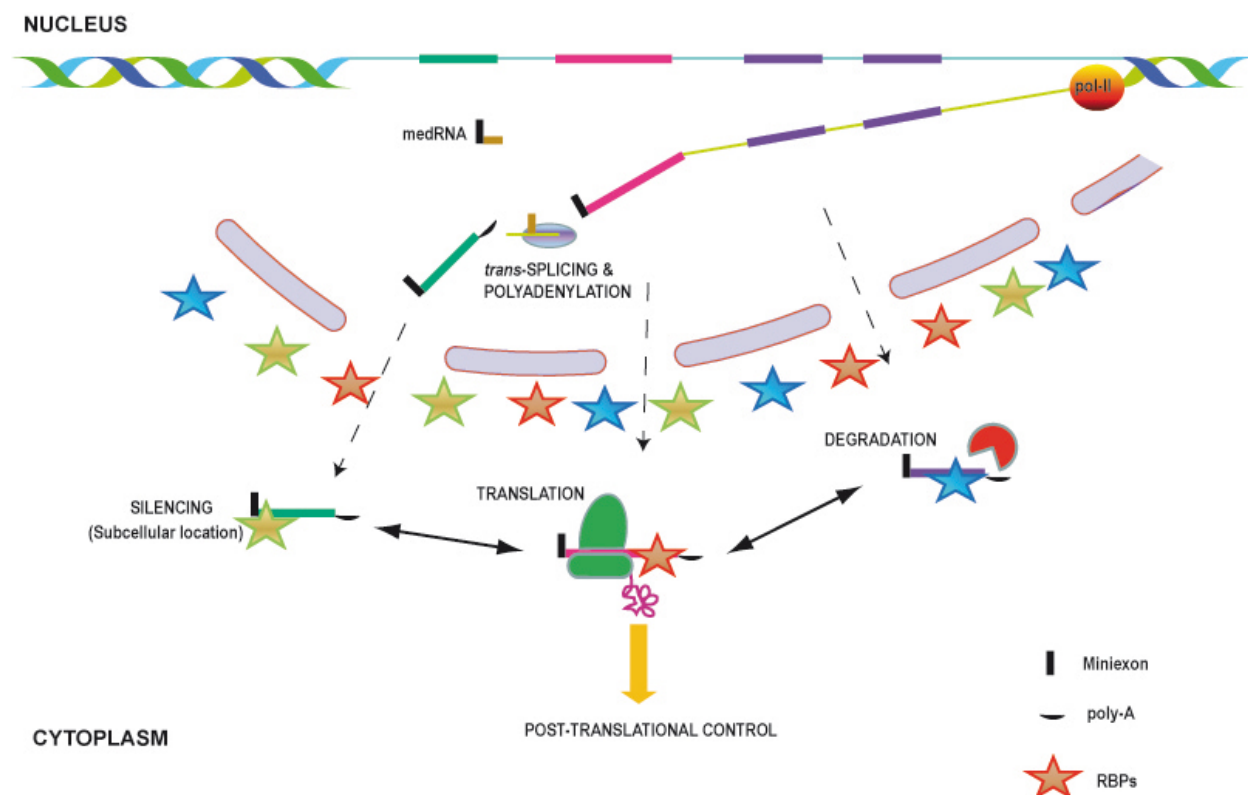
Figure 2. Transcription and processing of mRNAs in *Leishmania*. Transcription is polycistronic, and monocistronic RNAs are resolved by coupled *trans*-splicing and polyadenylation processes, yielding mRNAs containing a miniexon at its 5'-end and a poly-A tail at its 3'-end. When processing is complete, the mRNA is released from the site of transcription and is exported from the nucleus through the nuclear pores. At that point, some mRNA-binding proteins are stripped from the transcript, while others are removed only after export and yet others remain attached before and after export. Once in the cytoplasm, the mRNA must associate with proteins required for cytoplasmic transport, translation, degradation and anchoring. Finally, upon protein expression, protein function will be modulated by different post-translational mechanisms (protein modifications, differential cell sorting and degradation).

cytoplasmic transport (to specific subcellular location), translation, storage or degradation (Figure 2).

6. REGULATION OF mRNA STABILITY THROUGH *CIS*-ACTING ELEMENTS

Mature transcripts contain regulatory motifs mostly located in the 5'- and 3'-UTRs that modulate transcript abundance by specific interactions with RNA-binding proteins (RBPs). Thus, specific interactions between RBPs and RNA motifs (*cis*-elements) are involved in the control of mRNA transport, stability, intracellular destination, and translation efficiency (51).

Several studies in *Leishmania* have documented changes in message stability or degradation mediated by labile protein factors, which may be negative or positive regulators of mRNA stability. For example, the steady state level of a subset of *GP63* mRNAs, expressed primarily in logarithmic phase promastigotes, increased 16.5-fold after incubation in cycloheximide. Thus, a highly labile negative regulatory protein, such as an RNase, might be specifically targeting log *GP63* mRNAs for degradation (52). In

contrast, the temperature-dependent accumulation of the type-I class of *HSP70* transcripts in *L. infantum* is prevented by cycloheximide treatment, indicating that ongoing protein synthesis is required for transcript stabilization and suggesting that a labile protein factor is acting as a positive regulator during heat shock (41).

Leishmania transcripts possess UTRs longer than most of eukaryotes, being particularly true for the 3'-UTRs (13). This noteworthy feature is likely related to the preponderance of posttrancriptional regulation in this organism, since mRNA stabilization/degradation is dependent on protein factors that recognize sequence and/or structural motif often found in the 3'-UTR of transcripts (53). In *Leishmania*, a growing number of 3'-UTR elements have been reported to be implicated in controlling transcript stability. Thus, for example, the regulatory *cis*-element responsible for *HSP70* mRNA accumulation during heat shock was mapped at the 3'-UTR (54). Similarly, the stability and abundance of *Leishmania HSP83* transcripts, which increase at mammalian temperatures, are directed by *cis*-elements located at the 3'-UTR (42). The steady state levels of mRNAs for

GP63/MSP and gp46/PSA (two abundant membrane-anchored proteins on the surface of *Leishmania* promastigotes) experience a significant increase as promastigotes arise the stationary phase of growth, and the 3'-UTRs of these mRNAs are essential for this post-transcriptional regulation (55). The mature transcript derived from *A600-4* gene is eight-fold more abundant in *L. mexicana* amastigotes than promastigotes; it has been shown that the stage-specific expression is conferred by the 3'-UTR of the transcript (56).

The developmentally regulated gene family encoding the amastin surface proteins constitutes another interesting example illustrating the complexities of gene regulation in *Leishmania*. The majority of the members of this large gene family are specifically expressed in the intracellular amastigote stage of the parasite (57). It was shown that regions within the 3'-UTR of the amastin transcripts are involved in their developmental regulation (58). A remarkable finding was the identification of a 450-nt element that is highly conserved in the 3'-UTRs of the majority of the amastin transcripts (57). This conserved element is also present in a large number of *Leishmania* mRNAs, some of them are known to be also developmentally regulated in the mammalian-living form of the parasite (59). Recently, it was found that this regulatory element is part of the SIDER1 subfamily (60). SIDERs (short interspersed degenerated retroposons) are repetitive DNA elements derived from extinct retroposons. These elements, initially identified in the genome of *L. major* (60), have been found quite uniformly dispersed throughout the genomes of other *Leishmania* species (61, 62). Members of the other subfamily, SIDER2, are often located within the 3'-UTRs of genes and, for the experimentally analyzed cases, their presence is associated with decreased stability (and consequently lower steady state levels) of the LmSIDER2-containing transcripts (60). Hence, it has been postulated that these extinct retroposons have been recycled in *Leishmania* to accomplish large-scale modulation of post-transcriptional gene expression (11). A role of mobile elements and non-coding repetitive sequences, as modular parts of complex regulatory elements ensuring the coordinated expression of various mRNA species, has also been suggested from studies in other organisms (63, 64).

In most of the studies addressing the mapping of regulatory elements within the 3'-UTRs of differentially expressed genes, the results suggest that *cis*-elements must have complex structures, involving regions spanning several hundred nucleotides (42, 54, 55, 56). However, a few fortunate groups have been able to map regulatory elements in shorter regions of the 3'-UTRs. Thus, Mishra and co-workers (65) identified a regulatory element contained within 10-nt of the 3'-UTR of *L. mexicana* *PFR2C* gene that is necessary for the down-regulation of paraflagellar rod (PFR) transcripts in the mammalian stage. This element, termed PRE (for PFR regulatory element), acts as a negative regulatory element by destabilizing the *PFR2* mRNAs in amastigotes. The PFR protein is essential for flagellar motility in *Leishmania* promastigotes, but it is absent from the atrophied flagellum of amastigotes (66,

67). A search in the *L. major* genome database has revealed the presence of PRE element in genes other than *PFR* one; remarkably, the corresponding transcripts display also a promastigote-enriched accumulation (68). Another example is the octamer sequence (C/A)ATAGAA(G/A), which has been found by computational analysis in many S-phase expressed genes of *L. major*; its presence has been postulated to be involved in the mRNA accumulation observed during S-phase for some of the identified genes (69). This sequence element was previously identified at either the 5'- or 3'-UTRs of multiple cell-cycle regulated genes in *Crithidia fasciculata* (70), and it was involved in the regulation of the cell cycle-dependent accumulation of these mRNAs. Recently, a protein, named LdCSBP, which specifically binds to the octamer RNA sequence, has been identified in *L. donovani* (71). LdCSBP contains two Zn-finger motifs, probably responsible for binding to RNA, and two ubiquitin interacting domains, which can explain the observed ubiquitination of the protein.

7. CONTROL OF mRNA TRANSLATION

Both the global control of protein synthesis and mRNA-specific translational regulation represent key mechanisms of gene modulation. Furthermore, translation is the final step in the flow of the genetic information, and regulation at this level allows for an immediate and rapid response to changes in physiological conditions (72, 73). Upon export, not all mRNAs immediately enter the translationally active pool (Figure 2). Many are held instead in a translationally quiescent state awaiting either proper subcellular localization or some signal alerting that timing is now right to make protein. Translational control has been documented in a wide range of organisms and in many cellular processes, including the cellular response to stress and apoptosis, the regulation of cell growth and its coordination with cell division, and during differentiation and development (53, 72). Translational regulation plays a critical role during development and is in general mediated by cis-acting signals in the 3'UTR of target mRNAs and *trans*-acting RNA binding proteins (74).

The application of DNA microarray technologies to study gene expression by quantitation of mRNA levels has led to a somewhat surprising observation: only 0.2 to 5% of *Leishmania* genes are differentially expressed at the RNA level between the amastigote and promastigote life stages (75, 76, 77). Furthermore, these microarray analyses support the hypothesis that most of *Leishmania* genome is constitutively expressed in both life stages. On the contrary, proteomic analyses have revealed that up to 18% of the *Leishmania* proteins are differentially expressed between promastigote and amastigote stages (78, 79, 80, 81). In addition, stage-specific protein expression levels show a weak correlation with the corresponding mRNA levels (76, 80). These findings suggest that, in general, regulation of gene expression in *Leishmania* by controlling mRNA levels may be less relevant than regulation at the level of protein expression mediated by translational control, protein stability and post-translational modifications. However, this does not exclude that for particular genes regulation at the level of mRNA stability may be playing a key role.

Another interesting finding to note is that some genes that are regulated at the mRNA stability level in a given *Leishmania* species do not seem to be similarly regulated in other species (82).

For some genes in *Leishmania*, the regulation at the level of translation has been demonstrated as a particularly relevant mechanism. Thus, the regulation of expression of histone genes in *Leishmania* has been found to operate mainly at the translational level (83, 84). Histones forming the nucleosomal core (H2A, H2B, H3 and H4) are extremely conserved proteins, reflecting their importance in the organization of DNA in the eukaryotic nucleus. Given this functional association between DNA and histones, it is not unexpected that histone synthesis is tighly linked to cellular DNA replication (85). Most histone protein synthesis is restricted to the S-phase of the cell cycle, when they are required for assembly into nucleosomes with the newly replicated DNA. Expression of histone genes, in most eukaryotes, has multiple points of control, operating at both transcriptional and post-transcriptional levels (86). However, the regulation of histone expression in *Leishmania* parasites relies on a mechanism operating at the translational level, since neither transcription rates of histone genes nor transcript stability are affected by the inhibition of DNA synthesis (83). Nevertheless, the levels of histone synthesis increase when parasites enter the S-phase, and, along the cell cycle, drastic changes in the polysome profiles of histone mRNAs are observed. Thus, in the S-phase, histone mRNAs associate with ribosomes and poly-ribosomes, but in the G1-phase the histone transcripts are mainly found in ribosome-free fractions (84), suggesting the existence of a translational silencing of histone mRNAs at the non-S phases. Recently, it has been demonstrated the essential role played by the 3'-UTR of *histone H2A* genes in the cell cycle regulated translation of histone mRNAs in *Leishmania* (87).

Translational regulation has also been shown to be relevant for the expression of heat shock genes in *Leishmania*, at least for *HSP83* and *HSP70* genes, being the 3'-UTRs of these genes essential for the translational control (88, 89, 90). *Leishmania* promastigotes experience a drastic change in the environmental temperature when transmission to mammalian host occurs (Figure 1), and the heat-shock response and subsequent temperature-induced gene expression are considered paramount in the differentiation process (3, 91, 92). HSP70, the most evolutionarily conserved and ubiquitous heat shock protein, is encoded in different *Leishmania* species by six *HSP70* genes arranged in a head-to-tail tandem (93). All the genes are conserved at their 5'-UTRs and coding regions; only the *HSP70* gene located at the 3'-end of the tandem shows a 3'-UTR completely divergent relative to the 3'-UTR shared by the other five genes (41). For simplicity, genes 1 to 5 are known as *HSP70* type I (*HSP70-I*) genes whereas the gene 6 is referred as *HSP70-II* gene (89). The most abundant *HSP70* mRNAs derive from *HSP70-II* gene, but only transcripts derived from *HSP70-I* genes accumulate after heat shock (41). It was found that mRNAs derived from *HSP70-I* genes contain a *cis*-acting sequence which functions as a positive element that is responsible for the temperature-dependent accumulation of these transcripts (see above). However, when translational activity of *HSP70* mRNAs was analyzed by polysomal distribution, it was somewhat surprising to observe that the abundant *HSP70-II* mRNAs are translationally silent at normal growth temperatures, whereas the heat-inducible *HSP70-I* transcripts are translated at both normal and heat shock temperatures (89). Thus, *HSP70-II* mRNAs seem to be stored in a translational silent form during normal growth for the purpose of being rapidly translated when parasites encounter a stress situation and extra amount of HSP70s is required.

Preferential translation of *Leishmania HSP83* mRNAs during heat shock has been documented by several authors and analyzed in detail by Shapira's group in a recent work (90). In previous works, the 3'-UTR of *HSP83* mRNAs was shown to be essential for this translational regulation in different *Leishmania* species (42, 88). Recently, a polypyrimidine-rich region have mapped, by sequential deletions of the *HSP83* 3'-UTR, to be required, but not sufficient, for preferential translation of a reporter gene at mammalian-like temperatures. These authors propose that this regulatory sequence experiences structural changes at *Leishmania*-host temperatures, acting as a thermosensor and facilitating translation initiation of *HSP83* mRNAs at elevated temperatures (90).

Amastin is another *Leishmania* gene in which translational regulation has been demonstrated. As detailed above, amastins are surface proteins that are specifically expressed in the intracellular amastigote stage of the parasite (57). McNicoll and co-workers (94) showed that a 450-nucleotide element of the 3'-UTR (also found in several other *Leishmania* mRNAs, see above) stimulates translation of amastin mRNAs in response to heat shock, which is the main environmental change that the parasite encounters upon its entry into the mammalian host. Further analysis depicted a second region of ~ 100-nt (also in the 3'-UTR) which also activates translation in response to elevated temperatures. Both 3'-UTR regulatory elements have an additive effect on amastin mRNA translation (94). Since amastin mRNAs are expressed at very low levels in promastigotes, it has been suggested that regulation at the level of translation could provide a rapid and efficient way to ensure the production of the amastin surface proteins shortly following the entry of the parasite into macrophages after *Leishmania* transmission to the mammalian host (94).

LmxPK4, a mitogen-activated protein kinase kinase homologue, is present in the promastigote stage of *L. mexicana* and during *in vitro* differentiation from pro- to amastigotes but was not detectable in amastigotes despite the presence of mature mRNA in both life cycle stages. Thus, it represents another example for the regulation of gene expression at the translational level in trypanosomatids (95). In summary, translational control is emerging as an important regulatory mechanism in *Leishmania* and surely soon it will be demonstrated that the expression of many genes is regulated at this level. On the other hand, most attempts to define *cis*-elements in

Table 1. Regulatory RNA-binding proteins characterized in trypanomatids

Protein (GeneDB name)[a]	Organism	Functions and properties	Homologue in *Leishmania*[b]	References
TcUBP-1[c] (Tc00.1047053507093.229)	*T. cruzi*	Regulation of SMUG mucin mRNAs, binding to AU-rich elements (ARE) in mRNAs Binding to mRNAs coding for metabolic proteins	LmjF25.0490	102, 104
TcRBP3 (Tc00.1047053507093.250)	*T. cruzi*	Binding to ribosomal protein-encoding transcripts	LmjF25.0520	104
TbDRBD3[d] (Tb09.211.0560)	*T. brucei*	Binding to mRNAs coding for membrane proteins and metabolic enzymes	LmjF04.1170	106
TbZFP3 (Tb927.3.720)	*T. brucei*	Regulation of procyclin mRNAs Associated with the translational machinery	LmjF27.0130	107, 108
TbPUF1 (Tb927.10.4430)	*T. brucei*	Interaction with ESAG8 protein and possibly with *ESAG8* mRNA Preferentially bound to retroposon RNAs	LmjF36.0050	113, 115
TcPUF6[e] (Tc00.1047053508625.160)	*T. cruzi*	Down-regulation of several mRNAs Interaction with TcDhh1 (decapping activator)	LmjF36.0050	116
TbPUF9 (Tb927.1.2600)	*T. brucei*	Stabilization of transcripts coding for proteins involved in kinetoplast and cellular replication	LmjF20.1365	118
PTB1[d] (DRBD3) (Tb09.211.0560)	*T. brucei*	Homologue to human PTB (polypyrimidine tract binding protein) Regulation or mRNA stability and *trans*-splicing of a subset of transcripts	LmjF04.1170	120
PTB2 (DRBD4) (Tb11.01.5690)	*T. brucei*	Homologue to human PTB (polypyrimidine tract binding protein) Regulation or mRNA stability and *trans*-splicing of a subset of transcripts	LmjF32.0850	120

[a]Systematic names in the TriTryp databases (www.GeneDB.org), [b]The gene identification number in the *L. major* database (GeneDB) is indicated, [c]This protein belongs to a RBP family composed of at least six members that share similar primary structures (103), [d]Note that TbDRBD3 and PTB1 are the same protein, [e]TcPUF6 is orthologue to TbPUF1

Leishmania genes that are regulated at the translational level have led to the involvement of large regions in which different elements might exist and co-interacts (90, 94). It is likely that these findings are related to a complexity of regulatory mechanisms that has not been uncovered yet in this protozoan parasite. For example, mRNA subcellular location is emerging as a major mechanism of gene regulation in eukaryotes, allowing cells to establish functionally distinct compartments and structures (96). Remarkably, in these cases, the definition of the *cis*-elements responsible for localizing mRNAs has also turned to be difficult, and often extensive regions and multiple interplaying elements in the transcripts have been involved (reviewed in references 97 and 98). The reason may be found in the fact that probably there are many *trans*-acting factors interpreting the mRNA signals, and their combinations change during the life of an mRNA and according to its subcellular destinations.

In summary, evidence is accumulating that mRNA-specific regulation of the translation and degradation rates may be essential for proper control of gene expression in *Leishmania*. Such post-transcriptional control would be commonly mediated by proteins that bind in a sequence-specific fashion to regulatory elements located in the 3'-UTRs of mRNAs (see next section). However, the mechanisms by which these proteins either promote or inhibit translation and/or degradation of the bound mRNAs are poorly understood in general and for *Leishmania* in particular, being a clear challenge for the future.

8. *TRANS*-ACTING FACTORS AND THEIR INVOLVEMENT IN SPECIFIC REGULATORY MECHANISMS OF GENE EXPRESSION

Posttranscriptional processing, cellular localization, stabilization and degradation, and the specific translational rate of mRNAs are mediated by *trans*-acting

factors that both specifically and dynamically recognize and bind RNAs (99). Consistent with the predominance of post-transcriptional mechanisms of gene expression in *Leishmania*, and related trypanosomatids, a large number of putative RBPs are encoded in the genomes of these parasites (5, 100). However, even though the involvement of proteins in regulating stability and translation of particular mRNAs has been invoked (see above), few RNA-protein interactions have been documented in trypanosomatids, but none in *Leishmania* apart from the poly(A)-binding protein (101). Given the existence of high sequence conservation between the *trans*-acting proteins identified in *Trypanosoma* (*T. brucei* and *T. cruzi*) and their homologues in *Leishmania* (100), it is plausible that these proteins are playing similar roles in both genera of parasites. Hence, a description of the experimentally verified regulatory RBPs in *Trypanosoma* and related organisms was considered relevant for the purpose of this review (Table 1).

The first RBP with a specific regulatory function described in trypanosomatids was TcUBP-1 (102). This protein, characterized in *T. cruzi*, was identified by its specific recognition of an AU-rich element located in the 3'-UTR of mucin *SMUG* mRNAs. Since TcUBP-1 is developmentally regulated, it has been suggested that the protein might be involved in regulating the expression of U-rich sequence containing mRNAs during trypanosome differentiation (102). TcUBP-1 belongs to a RBP family composed of at least six members that share similar primary structures (103). In a recent study, a systematic identification of mRNA targets for TcUBP-1 and TcRBP3 (another member of the TcUBP-1 family) has shown that each one of the proteins bind preferentially a distinct subset of mRNAs. Thus, TcUBP-1 is preferentially associated with mRNAs encoding proteins involved in metabolism, whereas ribosomal protein-encoding transcripts were the largest group within TcRBP3-interacting transcripts (104). Remarkably, it has been described that during nutritional

stress both TcUBP-1 and TcRBP3 re-localize in large cytoplasmic granules containing other RBPs and polyadenylated mRNAs (105).

Another RBP, TbDRBD3, was initially identified because of its affinity for the 3'- UTR of mRNAs coding for the phosphoglycerate kinase B in *T. brucei*. Additional experiments showed that this protein interacts with a group of mRNAs encoding membrane proteins and intermediate metabolism enzymes (106). All TbDRBD3-regulated mRNAs contain the UAUUUUUU element and these transcripts result protected from degradation after binding to TbDRBD3. There exists a homologue protein to TbDRBD3 in the *L. major* database (i.e., LmjF04.1170) and it should be interesting to address whether or not the *Leishmania* protein is involved in the regulation of an equivalent set of mRNAs.

Recently, it has been identified a protein (TbZFP3) that associates with the 3'-UTR of some procyclin mRNAs, which encode the major surface proteins of *T. brucei* parasites in the tsetse fly (107). In a previous work, it was found that TbZFP3 interacts with the translational machinery, and that its association with polyribosomes is developmentally regulated (108). Remarkably, although procyclin mRNAs are not present in *Leishmania*, a well conserved homologue to *TbZFP3* gene is annotated in the *Leishmania* databases (Table 1).

Most RBPs, in trypanosomes and other organisms, are not exclusive for an mRNA species, but they associate with subsets of mRNAs coding for functionally related proteins. TcUBP-1 and TcRBP3 are adequate examples (see above). These observations has led to coin the term of "posttranscriptional operons", which reflect the idea that multiple mRNAs are regulate in a coordinate manner by specific RBPs (51). This is well exemplified by a family of RBPs, the PUF family in yeast, where the different members of the family associate to mRNAs encoding components of specific cellular processes. Thus, for example, PUF1 and PUF2 bind mRNAs coding for membrane-associated proteins, PUF3 binds to cytoplasmic mRNAs coding for mitochondrial proteins, PUF4 interacts with mRNAs encoding nucleolar ribosomal RNA-processing factors, and PUF5 is associated with mRNAs encoding chromatin modifiers and components of the spindle pole body (109).

The PUF proteins constitute an evolutionarily conserved protein family named after Pumilio (*Drosophila*) and FBF (Fem-3 mRNA-binding Factor, *Caenorhabditis elegans*). This class of RBPs regulates translation and RNA stability by binding to specific sequences in the 3'-UTR of target mRNAs (110, 111). Trypanosomatids contain a relatively high number of proteins showing the characteristic structural motifs of PUF family. Thus, the PUF family in *T. cruzi* and *T. brucei* is composed of 10 members (112, 113), whereas a recent duplication affecting *PUF9* gene has increased the PUF family in *Leishmania* to 11 members (114). Until now, few studies on the functional implications of PUF proteins in trypanosomatids have been published, but none in *Leishmania*. *T. brucei* PUF1

(TbPUF1) was identify as a cytoplasmic ESAG8-interacting protein (115). Expression-site-associated genes (ESAGs) are co-transcribed with *VSG* (variant surface glycoproteins) genes from the same upstream promoter. Overexpression of TbPUF1 leads to accumulation of both *ESAG8* mRNA and protein, suggesting that TbPUF1 might be regulating stability of *ESAG8* mRNAs (115). More recently, the purification of RNAs bound to the TAP-tagged LbPUF1 in procyclic trypanosomes and their analysis using microarrays showed that LbPUF1 interacts with RNAs derived from retroposon elements (113). A similar experimental approach was followed to identify RNA targets of the TbPUF1 ortholog in *T. cruzi* (named TcPUF6!), leading to the identification of a different set of RNA targets (116). Remarkably, the abundance of these putative RNA targets decreased in TcPUF6-overexpressing parasites, suggesting that TcPUF6 enhances the degradation of its associated mRNAs. Interestingly, in line with this hypothesis, it was demonstrated an interaction betweenTcPUF6 and the decapping activator TcDhh1 (116). TcDhh1 has been found in P body-like structures in *T. cruzi* (117). More recently, Clayton and colleagues (118) have described that *T. brucei* PUF9 (TbPUF9) regulates mRNAs for proteins involved in replicative processes over the cell cycle. It has been suggested that LbPUF9-associated mRNAs may constitute a post-transcriptional regulon, encoding proteins involved in replicative processes occurring at early G2 phase (118).

Another interesting group of RNA binding proteins are the polypyrimidine tract binding proteins (PTBs), also known as hnRNPI, which act at multiple steps during mRNA biogenesis (119). Two *T. brucei* RNA binding proteins named PTB1 and PTB2, because of their resemblance to mammalian PTBs, have been identified and characterized (120). It was found that PTB1 and PTB2 are essential for *trans*-splicing of transcripts carrying C rich polypyrimidine tracts. In addition, as occurs with PTB in mammals, PTB1 and PTB2 regulate mRNA stability, affecting each one the stability of distinct sets of mRNAs. Recently, it was suggested that DRBD4 (=PTB2) in *T. cruzi* functions in masking *trans*-splicing/polyadenylation signals, leading to the production of dicistronic mRNAs, since DRBD4 was found to bind dicistronic mRNAs present in the cytoplasm (121). Thus, it has been proposed by Frasch and co-workers that splice-site skipping might be another posttranscriptional mechanism in trypanosomes to regulate gene expression through which precursor RNAs can be stored in a "translational latency state" for further processing into a mature transcript when required for the parasite (121).

9. REGULATION AT THE POST-TRANSLATIONAL LEVEL

Post-translational modifications (PTMs) of proteins have a relevant contribution to the control of gene expression. Thus, PTMs have a direct effect on stability and turnover, subcellular localization, and activity of proteins. Phosphorylation, methylation, acetylation, glycosylation, N-terminal pyroglutamylaton, deamidation, and tryptophan oxidation are PTMs described to occur in

Leishmania and related trypanosomatids (122). Proteomic studies based on the separation of *Leishmania* proteins by two-dimensional (2-D) gel electrophoresis have shown that many proteins are present in multiple spots, suggesting that PTMs are frequent and extensive in this organism (80, 81). A high-throughput analysis of PTMs in both axenic promastigotes and amastigotes of *L. donovani* has been carried by the Zilberstein's group (123). A total of 16 phosphorylated, 20 methylated, 26 acetylated, and 13 glycosylated distinct proteins were detected. In addition, it was found that the relative abundance of these PTMs varies during promastigote to amastigote differentiation. In a recent report, Morales and co-workers (124) have shown that protein chaperones, including isoforms of HSP83/90 and various HSP70 family members, are more phosphorylated in amastigotes than in promastigotes. Furthermore, they demonstrated that phosphorylation of STI1 (a cochaperone for HSP90 and HSP70) at two specific serine residues is essential for parasite viability (124).

The expression and biosynthesis of the major surface metalloprotease, GP63, in *Leishmania* have been shown to be regulated also at the post-translational level, and it serves to illustrate the complexity of post-transcriptional mechanisms. GP63 is synthesized in the endoplasmic reticulum and transported to the plasma membrane (125). During trafficking, a signal peptide and a pro-peptide at the N-terminus are cleaved (126), a glycosylphosphatidylinositol (GPI) anchor is attached near the C-terminus (127), and N-glycosylation can occur at several sites (127). Although an important fraction of GP63 becomes surface localized, the protein is also released into the extracellular medium (128). Additionally, 2-D gel electrophoresis and immunoblots have shown the existence of more than 10 GP63 isoforms in stationary phase promastigotes of *L. chagasi*, distributed between pIs of 5.2-6.1 and masses of 58-63 kDa, even though some of the isoforms are the products of different genes (129). Interestingly, the GP63 isoforms are differentially expressed in different promastigote growth phases, and attenuation of parasites by long-term *in vitro* cultivation influences GP63 isoform expression (130). Taking into account these findings, it has been suggested that the expression of GP63 as a functional protease might be regulated and modulated by different PTM pathways.

The importance of post-translational regulation of protein abundance in *Leishmania* has also been underlined by studies on the cell cycle-dependent expression of *L. major* Kin-13 kinesin (LmjKIN13-1) (131). Kinesins are found in all eukaryotes and are involved in many cellular processes by their ability to modulate interactions with microtubules. LmjKIN13-1 remains strictly localized in the nucleus and its expression is cell cycle-regulated (131). The protein is expressed before the onset of mitosis (G2+M phase) and it disappears after the end of telophase and cytokinesis. Current experimental evidence suggests a role for the ubiquitin/proteasome pathway in the cell cycle regulation of this protein (131).

The growth phase regulation of the main folate transporter of *L. infantum* is another example of the importance of post-translational regulation in this organism (132). *Leishmania* parasites are auxotroph for folate and need to import this essential substrate from the external medium. For this purpose, the parasites possess specific transporters involved in folate uptake, being FT1 the main folate transporter. Remarkably, FT1 is found at the plasma membrane in the logarithmic growth phase of the parasite, but the protein is retargeted intracellularly when the parasite reaches the stationary growth phase (132). This redistribution from the plasma membrane to an intracellular compartment seems to be accompanied by a specific degradation of the FT1 protein. Similarly, expression of the purine nucleobase transporter NT3 is profoundly downregulated at the protein but not the mRNA level in stationary phase compared with logarithmic phase promastigotes in *L. major* (133). *Leishmania* parasites are not able to synthesize purines *de novo* and need to uptake preformed purines from their hosts (134). The NT3 transporter, that is a high affinity transporter for purine nucleobases, also mediates the uptake of the anti-leishmanial drug allopurinol (133).

In summary, several studies are revealing that PTMs may be highly widespread in the *Leishmania* proteome and of paramount importance to stage differentiation and other aspects of the parasite biology.

10. CONCLUSIONS AND PERSPECTIVES

Post-transcriptional regulation of mRNAs is increasingly recognized as a central pathway controlling gene expression in eukaryotes, being particularly important in *Leishmania* and other trypanosomatids, where little or no evidence for regulation of transcription initiation exits. Thus, these organisms represent outstanding examples of the importance of mRNA metabolism in the regulation of gene expression. Considerable progress has been made in understanding gene organization and expression in *Leishmania*. In particular, the determination of genome sequence for several *Leishmania* species has being invaluable for the knowledge of many relevant aspects of the molecular biology of these parasites. It has been demonstrated that regulation of gene expression operate mainly by post-transcriptional mechanisms and that the regulation of many differentially expressed genes is directed by their 3'-UTRs.

Global proteomics and transcriptomic studies in different *Leishmania* species have demonstrated a low degree of differential mRNA expression but a higher degree of regulation at the translational and post-translarional levels. In addition, PTMs of proteins are likely to play a key role in the regulation of metabolism and development of this microorganism, and future in-depth analyses are required.

Bioinformatics surveys on genome databases have shown that the number of RBPs encoded in the *Leishmania* genome is large. Now, it is necessary to identify the transcripts that interact with each specific protein, and understand how RBPs are assembled in regulatory pathways. Although RBPs have not been

functionally characterized in *Leishmania* to date, fortunately the number of RBPs studied in other related trypanosomatids (*T. brucei* and *T. cruzi*) is growing (Table 1).

A major conclusion emerging from all these studies on gene expression in *Leishmania*, and when comparing with gene regulation in other organisms, is that no universal control mechanism exists. Rather, each organism seems to pick from a palette of potential regulatory mechanisms (storage of masked mRNAs, transcriptional control, translational control, mRNA stabilities, etc.) its own blend of solutions to the same basic problem: to supply adequately the specific, temporal demand of its growing and differentiating cells for proteins (135). How such multiple regulatory mechanisms are effectuated and coordinated in molecular terms is our challenge for the future.

The unique features of the molecular biology and a better understanding of the way gene expression is regulated in *Leishmania* offer avenues for exploring new therapeutic and preventive strategies to control leishmaniasis, a disease that continues affecting millions of people worldwide.

11. ACKNOWLEDGMENTS

12. REFERENCES

1. Dujardin, J. C., L. Campino, C. Cañavate, J. P. Dedet, L. Gradoni, K. Soteriadou, A. Mazeris, Y. Ozbel & M. Boelaert: Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. *Emerg Infect Dis*, 14, 1013-8 (2008)

2. Bates, P. A.: Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. *Int J Parasitol*, 37, 1097-106 (2007)

3. Zilberstein, D. & M. Shapira: The role of pH and temperature in the development of *Leishmania* parasites. *Annu Rev Microbiol*, 48, 449-70 (1994)

4. Sogin, M. L., H. J. Elwood & J. H. Gunderson: Evolutionary diversity of eukaryotic small-subunit rRNA genes. *Proc Natl Acad Sci U S A*, 83, 1383-1387 (1986)

5. Ivens, A. C., C. S. Peacock, E. A. Worthey, L. Murphy, G. Aggarwal, M. Berriman, E. Sisk, M. A. Rajandream, E. Adlem, R. Aert, A. Anupama, Z. Apostolou, P. Attipoe, N. Bason, C. Bauser, A. Beck, S. M. Beverley, G. Bianchettin, K. Borzym, G. Bothe, C. V. Bruschi, M. Collins, E. Cadag, L. Ciarloni, C. Clayton, R. M. Coulson, A. Cronin, A. K. Cruz, R. M. Davies, J. De Gaudenzi, D. E. Dobson, A. Duesterhoeft, G. Fazelina, N. Fosker, A. C. Frasch, A. Fraser, M. Fuchs, C. Gabel, A. Goble, A. Goffeau, D. Harris, C. Hertz-Fowler, H. Hilbert, D. Horn, Y. Huang, S. Klages, A. Knights, M. Kube, N. Larke, L. Litvin, A. Lord, T. Louie, M. Marra, D. Masuy, K. Matthews, S. Michaeli, J. C. Mottram, S. Muller-Auer, H. Munden, S. Nelson, H. Norbertczak, K. Oliver, S. O'Neil, M. Pentony, T. M. Pohl, C. Price, B. Purnelle, M. A. Quail, E. Rabbinowitsch, R. Reinhardt, M. Rieger, J. Rinta, J. Robben, L. Robertson, J. C. Ruiz, S. Rutter, D. Saunders, M. Schafer, J. Schein, D. C. Schwartz, K. Seeger, A. Seyler, S. Sharp, H. Shin, D. Sivam, R. Squares, S. Squares, V. Tosato, C. Vogt, G. Volckaert, R. Wambutt, T. Warren, H. Wedler, J. Woodward, S. Zhou, W. Zimmermann, D. F. Smith, J. M. Blackwell, K. D. Stuart, B. Barrell & P. J. Myler: The Genome of the Kinetoplastid Parasite, *Leishmania major*. *Science*, 309, 436-42 (2005)

6. El-Sayed, N. M., P. J. Myler, D. C. Bartholomeu, D. Nilsson, G. Aggarwal, A. N. Tran, E. Ghedin, E. A. Worthey, A. L. Delcher, G. Blandin, S. J. Westenberger, E. Caler, G. C. Cerqueira, C. Branche, B. Haas, A. Anupama, E. Arner, L. Aslund, P. Attipoe, E. Bontempi, F. Bringaud, P. Burton, E. Cadag, D. A. Campbell, M. Carrington, J. Crabtree, H. Darban, J. F. da Silveira, P. de Jong, K. Edwards, P. T. Englund, G. Fazelina, T. Feldblyum, M. Ferella, A. C. Frasch, K. Gull, D. Horn, L. Hou, Y. Huang, E. Kindlund, M. Klingbeil, S. Kluge, H. Koo, D. Lacerda, M. J. Levin, H. Lorenzi, T. Louie, C. R. Machado, R. McCulloch, A. McKenna, Y. Mizuno, J. C. Mottram, S. Nelson, S. Ochaya, K. Osoegawa, G. Pai, M. Parsons, M. Pentony, U. Pettersson, M. Pop, J. L. Ramirez, J. Rinta, L. Robertson, S. L. Salzberg, D. O. Sanchez, A. Seyler, R. Sharma, J. Shetty, A. J. Simpson, E. Sisk, M. T. Tammi, R. Tarleton, S. Teixeira, S. Van Aken, C. Vogt, P. N. Ward, B. Wickstead, J. Wortman, O. White, C. M. Fraser, K. D. Stuart & B. Andersson: The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science*, 309, 409-15 (2005)

7. Berriman, M., E. Ghedin, C. Hertz-Fowler, G. Blandin, H. Renauld, D. C. Bartholomeu, N. J. Lennard, E. Caler, N. E. Hamlin, B. Haas, U. Bohme, L. Hannick, M. A. Aslett, J. Shallom, L. Marcello, L. Hou, B. Wickstead, U. C. Alsmark, C. Arrowsmith, R. J. Atkin, A. J. Barron, F. Bringaud, K. Brooks, M. Carrington, I. Cherevach, T. J. Chillingworth, C. Churcher, L. N. Clark, C. H. Corton, A. Cronin, R. M. Davies, J. Doggett, A. Djikeng, T. Feldblyum, M. C. Field, A. Fraser, I. Goodhead, Z. Hance, D. Harper, B. R. Harris, H. Hauser, J. Hostetler, A. Ivens, K. Jagels, D. Johnson, J. Johnson, K. Jones, A. X. Kerhornou, H. Koo, N. Larke, S. Landfear, C. Larkin, V. Leech, A. Line, A. Lord, A. Macleod, P. J. Mooney, S. Moule, D. M. Martin, G. W. Morgan, K. Mungall, H. Norbertczak, D. Ormond, G. Pai, C. S. Peacock, J. Peterson, M. A. Quail, E. Rabbinowitsch, M. A. Rajandream, C. Reitter, S. L. Salzberg, M. Sanders, S. Schobel, S. Sharp, M. Simmonds, A. J. Simpson, L. Tallon,

C. M. Turner, A. Tait, A. R. Tivey, S. Van Aken, D. Walker, D. Wanless, S. Wang, B. White, O. White, S. Whitehead, J. Woodward, J. Wortman, M. D. Adams, T. M. Embley, K. Gull, E. Ullu, J. D. Barry, A. H. Fairlamb, F. Opperdoes, B. G. Barrell, J. E. Donelson, N. Hall, C. M. Fraser, S. E. Melville & N. M. El-Sayed: The genome of the African trypanosome *Trypanosoma brucei. Science*, 309, 416-22 (2005)

8. Martinez-Calvillo, S., S. Yan, D. Nguyen, M. Fox, K. Stuart & P. J. Myler: Transcription of *Leishmania major* Friedlin chromosome 1 initiates in both directions within a single region. *Mol. Cell*, 11, 1291-1299 (2003)

9. Liang, X. H., A. Haritan, S. Uliel & S. Michaeli: trans and cis splicing in trypanosomatids: mechanism, factors, and regulation. *Eukaryot Cell*, 2, 830-40 (2003)

10. Clayton, C. & M. Shapira: Post-transcriptional regulation of gene expression in trypanosomes and leishmanias. *Mol Biochem Parasitol*, 156, 93-101 (2007)

11. Haile, S. & B. Papadopoulou: Developmental regulation of gene expression in trypanosomatid parasitic protozoa. *Curr Opin Microbiol*, 10, 569-77 (2007)

12. Clayton, C. E.: Life without transcriptional control? From fly to man and back again. *Embo J*, 21, 1881-8. (2002)

13. Stiles, J. K., P. I. Hicock, P. H. Shah & J. C. Meade: Genomic organization, transcription, splicing and gene regulation in *Leishmania. Ann Trop Med Parasitol*, 93, 781-807. (1999)

14. Papadopoulou, B., M. Muller, A. Rochette, F. McNicoll, C. Dumas & C. Chow: Regulation of gene expression in *Leishmania* throughout a complex digenetic life cycle. In: *Leishmania*, after the genome (P.J. Myler and N. Fasel, eds), Caister Academic Press, Norfolk, UK (2008)

15. Peacock, C. S., K. Seeger, D. Harris, L. Murphy, J. C. Ruiz, M. A. Quail, N. Peters, E. Adlem, A. Tivey, M. Aslett, A. Kerhornou, A. Ivens, A. Fraser, M. A. Rajandream, T. Carver, H. Norbertczak, T. Chillingworth, Z. Hance, K. Jagels, S. Moule, D. Ormond, S. Rutter, R. Squares, S. Whitehead, E. Rabbinowitsch, C. Arrowsmith, B. White, S. Thurston, F. Bringaud, S. L. Baldauf, A. Faulconbridge, D. Jeffares, D. P. Depledge, S. O. Oyola, J. D. Hilley, L. O. Brito, L. R. O. Tosi, B. Barrell, A. K. Cruz, J. C. Mottram, D. F. Smith & M. Berriman: Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nat Genet*, 39, 839-47 (2007)

16. Martinez-Calvillo, S., D. Nguyen, K. Stuart & P. J. Myler: Transcription initiation and termination on *Leishmania major* chromosome 3. *Eukaryot Cell*, 3, 506-17 (2004)

17. Martinez-Calvillo, S., J. C. Vizuet-de-Rueda, L. E. Florencio-Martinez, R. G. Manning-Cela & E. E. Figueroa-Angulo: Gene expression in trypanosomatid parasites. *J Biomed Biotechnol*, 2010, 525241 (2010)

18. Moore, M. J.: From birth to death: the complex lives of eukaryotic mRNAs. *Science*, 309, 1514-8 (2005)

19. Thomas, S., A. Green, N. R. Sturm, D. A. Campbell & P. J. Myler: Histone acetylations mark origins of polycistronic transcription in *Leishmania major. BMC Genomics*, 10, 152 (2009)

20. Siegel, T. N., D. R. Hekstra, L. E. Kemp, L. M. Figueiredo, J. E. Lowell, D. Fenyo, X. Wang, S. Dewell & G. A. M. Cross: Four histone variants mark the boundaries of polycistronic transcription units in *Trypanosoma brucei. Genes Dev*, 23, 1063-76 (2009)

21. Panton, L. J., R. B. Tesh, K. C. Nadeau & S. M. Beverley: A test for genetic exchange in mixed infections of *Leishmania major* in the sand fly *Phlebotomus papatasi. J Protozool*, 38, 224-8 (1991)

22. Chauhan, S. C. & R. Madhubala: Glyoxalase I gene deletion mutants of *Leishmania donovani* exhibit reduced methylglyoxal detoxification. *PLoS One*, 4, e6805 (2009)

23. Mukherjee, A., G. Roy, C. Guimond & M. Ouellette: The gamma-glutamylcysteine synthetase gene of *Leishmania* is essential and involved in response to oxidants. *Mol Microbiol*, 74, 914-27 (2009)

24. Silvestre, R., A. Cordeiro-Da-Silva, N. Santarem, B. Vergnes, D. Sereno & A. Ouaissi: SIR2-deficient *Leishmania infantum* induces a defined IFN-gamma/IL-10 pattern that correlates with protection. *J Immunol*, 179, 3161-70 (2007)

25. Beverley, S. M.: Gene amplification in *Leishmania. Annu Rev Microbiol*, 45, 417-44 (1991)

26. Segovia, M. & G. Ortiz: LD1 amplifications in *Leishmania. Parasitol. Today*, 13, 342-348 (1997)

27. Dujardin, J. C.: Structure, dynamics and function of *Leishmania* genome: resolving the puzzle of infection, genetics and evolution? *Infect Genet Evol*, 9, 290-7 (2009)

28. Leprohon, P., D. Legare, F. Raymond, E. Madore, G. Hardiman, J. Corbeil & M. Ouellette: Gene expression modulation is associated with gene amplification, supernumerary chromosomes and chromosome loss in antimony-resistant *Leishmania infantum. Nucleic Acids Res*, 37, 1387-99 (2009)

29. Requena, J. M., M. Soto, L. Quijada & C. Alonso: Genes and chromosomes of *Leishmania infantum. Mem Inst Oswaldo Cruz*, 92, 853-8. (1997)

30. Inga, R., S. De Doncker, J. Gomez, M. Lopez, R. Garcia, D. Le Ray, J. Arevalo & J. C. Dujardin: Relation between variation in copy number of ribosomal RNA encoding genes and size of harbouring chromosomes in *Leishmania* of subgenus Viannia. *Mol Biochem Parasitol*, 92, 219-28. (1998)

31. Guerbouj, S., I. Guizani, N. Speybroeck, D. Le Ray & J. C. Dujardin: Genomic polymorphism of Leishmania infantum: a relationship with clinical pleomorphism? *Infection Genetics Evolution*, 1, 49-59 (2001)

32. Victoir, K., J. C. Dujardin, S. de Doncker, D. C. Barker, J. Arevalo, R. Hamers & D. Le Ray: Plasticity of gp63 gene organization in *Leishmania* (Viannia) *braziliensis* and *Leishmania* (Viannia) *peruviana*. *Parasitology*, 111, 265-73. (1995)

33. Soto, M., J. M. Requena, M. Garcia, L. C. Gomez, I. Navarrete & C. Alonso: Genomic organization and expression of two independent gene arrays coding for two antigenic acidic ribosomal proteins of *Leishmania*. *J Biol Chem*, 268, 21835-43. (1993)

34. Soto, M., J. M. Requena & C. Alonso: Isolation, characterization and analysis of the expression of the *Leishmania* ribosomal PO protein genes. *Mol Biochem Parasitol*, 61, 265-74. (1993)

35. Garcia-Estrada, C., Y. Perez-Pertejo, D. Ordoñez, R. Balaña-Fouce & R. M. Reguera: Analysis of genetic elements regulating the methionine adenosyltransferase gene in *Leishmania infantum*. *Gene*, 389, 163-73 (2007)

36. Darlyuk, I., A. Goldman, S. C. Roberts, B. Ullman, D. Rentsch & D. Zilberstein: Arginine homeostasis and transport in the human pathogen *Leishmania donovani*. *J Biol Chem*, 284, 19800-7 (2009)

37. Meade, J. C., K. M. Hudson, S. L. Stringer & J. R. Stringer: A tandem pair of *Leishmania donovani* cation transporting ATPase genes encode isoforms that are differentially expressed. *Mol Biochem Parasitol*, 33, 81-91 (1989)

38. Muller, M. & B. Papadopoulou: Stage-specific expression of the glycine cleavage complex subunits in *Leishmania infantum*. *Mol Biochem Parasitol*, 170, 17-27 (2010)

39. Ramamoorthy, R., K. G. Swihart, J. J. McCoy, M. E. Wilson & J. E. Donelson: Intergenic regions between tandem gp63 genes influence the differential expression of gp63 RNAs in *Leishmania chagasi* promastigotes. *J Biol Chem*, 270, 12133-9. (1995)

40. Coulson, R. M., V. Connor, J. C. Chen & J. W. Ajioka: Differential expression of *Leishmania major* beta-tubulin genes during the acquisition of promastigote infectivity. *Mol Biochem Parasitol*, 82, 227-36. (1996)

41. Quijada, L., M. Soto, C. Alonso & J. M. Requena: Analysis of post-transcriptional regulation operating on transcription products of the tandemly linked *Leishmania infantum* hsp70 genes. *J Biol Chem*, 272, 4493-9. (1997)

42. Zilka, A., S. Garlapati, E. Dahan, V. Yaolsky & M. Shapira: Developmental Regulation of Heat Shock Protein 83 in *Leishmania*. *J Biol Chem*, 276, 47922-9. (2001)

43. Soto, M., L. Quijada, R. Larreta, S. Iborra, C. Alonso & J. M. Requena: *Leishmania infantum* possesses a complex family of histone H2A genes: structural characterization and analysis of expression. *Parasitology*, 127, 95-105. (2003)

44. Yao, C., J. E. Donelson & M. E. Wilson: The major surface protease (MSP or GP63) of *Leishmania* sp. Biosynthesis, regulation of expression, and function. *Mol Biochem Parasitol*, 132, 1-16. (2003)

45. LeBowitz, J. H., H. Q. Smith, L. Rusche & S. M. Beverley: Coupling of poly(A) site selection and trans-splicing in *Leishmania*. *Genes Dev*, 7, 996-1007. (1993)

46. Agabian, N.: Trans splicing of nuclear pre-mRNAs. *Cell*, 61, 1157-60. (1990)

47. Requena, J. M., L. Quijada, M. Soto & C. Alonso: Conserved nucleotides surrounding the trans-splicing acceptor site and the translation initiation codon in *Leishmania* genes. *Exp Parasitol*, 103, 78-81. (2003)

48. Charest, H., W. W. Zhang & G. Matlashewski: The developmental expression of *Leishmania donovani* A2 amastigote- specific genes is post-transcriptionally mediated and involves elements located in the 3'-untranslated region. *J Biol Chem*, 271, 17081-90. (1996)

49. Brooks, D. R., H. Denise, G. D. Westrop, G. H. Coombs & J. C. Mottram: The stage-regulated expression of *Leishmania mexicana* CPB cysteine proteases is mediated by an intercistronic sequence element. *J Biol Chem*, 276, 47061-9. (2001)

50. Lamontagne, J. & B. Papadopoulou: Developmental regulation of spliced leader RNA gene in *Leishmania donovani* amastigotes is mediated by specific polyadenylation. *J Biol Chem*, 274, 6602-9. (1999)

51. Keene, J. D.: RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet*, 8, 533-43 (2007)

52. Wilson, M. E., K. E. Paetz, R. Ramamoorthy & J. E. Donelson: The effect of ongoing protein synthesis on the steady state levels of Gp63 RNAs in *Leishmania chagasi*. *J Biol Chem*, 268, 15731-6. (1993)

53. Mata, J., S. Marguerat & J. Bahler: Post-transcriptional control of gene expression: a genome-wide perspective. *Trends Biochem Sci*, 30, 506-14 (2005)

54. Quijada, L., M. Soto, C. Alonso & J. M. Requena: Identification of a putative regulatory element in the 3'-untranslated region that controls expression of HSP70 in *Leishmania infantum*. *Mol Biochem Parasitol*, 110, 79-91. (2000)

55. Myung, K. S., J. K. Beetham, M. E. Wilson & J. E. Donelson: Comparison of the Post-transcriptional Regulation of the mRNAs for the Surface Proteins PSA

(GP46) and MSP (GP63) of *Leishmania chagasi*. *J Biol Chem*, 277, 16489-97. (2002)

56. Murray, A., C. Fu, G. Habibi & W. R. McMaster: Regions in the 3' untranslated region confer stage-specific expression to the *Leishmania mexicana* a600-4 gene. *Mol Biochem Parasitol*, 153, 125-32 (2007)

57. Rochette, A., F. McNicoll, J. Girard, M. Breton, E. Leblanc, M. G. Bergeron & B. Papadopoulou: Characterization and developmental gene regulation of a large gene family encoding amastin surface proteins in *Leishmania* spp. *Mol Biochem Parasitol*, 140, 205-20 (2005)

58. Wu, Y., Y. El Fakhry, D. Sereno, S. Tamar & B. Papadopoulou: A new developmentally regulated gene family in *Leishmania* amastigotes encoding a homolog of amastin surface proteins. *Mol Biochem Parasitol*, 110, 345-57. (2000)

59. Boucher, N., Y. Wu, C. Dumas, M. Dube, D. Sereno, M. Breton & B. Papadopoulou: A common mechanism of stage-regulated gene expression in *Leishmania* mediated by a conserved 3'-untranslated region element. *J Biol Chem*, 277, 19511-20. (2002)

60. Bringaud, F., M. Muller, G. C. Cerqueira, M. Smith, A. Rochette, N. M. A. El-Sayed, B. Papadopoulou & E. Ghedin: Members of a large retroposon family are determinants of post-transcriptional gene expression in *Leishmania*. *PLoS Pathog*, 3, 1291-307 (2007)

61. Requena, J. M., C. Folgueira, M. C. Lopez & M. C. Thomas: The SIDER2 elements, interspersed repeated sequences that populate the *Leishmania* genomes, constitute subfamilies showing chromosomal proximity relationship. *BMC Genomics*, 9, 263 (2008)

62. Smith, M., F. Bringaud & B. Papadopoulou: Organization and evolution of two SIDER retroposon subfamilies and their impact on the *Leishmania* genome. *BMC Genomics*, 10, 240 (2009)

63. Vidal, F., E. Mougneau, N. Glaichenhaus, P. Vaigot, M. Darmon & F. Cuzin: Coordinated posttranscriptional control of gene expression by modular elements including Alu-like repetitive sequences. *Proc Natl Acad Sci U S A*, 90, 208-12. (1993)

64. Chen, L. L., J. N. DeCerbo & G. G. Carmichael: Alu element-mediated gene silencing. *Embo J*, 27, 1694-705 (2008)

65. Mishra, K. K., T. R. Holzer, L. L. Moore & J. H. LeBowitz: A negative regulatory element controls mRNA abundance of the *Leishmania mexicana* Paraflagellar rod gene PFR2. *Eukaryot Cell*, 2, 1009-17 (2003)

66. Bastin, P., T. Sherwin & K. Gull: Paraflagellar rod is vital for trypanosome motility. *Nature*, 391, 548. (1998)

67. Santrich, C., L. Moore, T. Sherwin, P. Bastin, C. Brokaw, K. Gull & J. H. LeBowitz: A motility function for the paraflagellar rod of *Leishmania* parasites revealed by PFR-2 gene knockouts. *Mol Biochem Parasitol*, 90, 95-109. (1997)

68. Holzer, T. R., K. K. Mishra, J. H. LeBowitz & J. D. Forney: Coordinate regulation of a family of promastigote-enriched mRNAs by the 3'UTR PRE element in *Leishmania mexicana*. *Mol Biochem Parasitol*, 157, 54-64 (2008)

69. Zick, A., I. Onn, R. Bezalel, H. Margalit & J. Shlomai: Assigning functions to genes: identification of S-phase expressed genes in *Leishmania major* based on post-transcriptional control elements. *Nucleic Acids Res*, 33, 4235-42 (2005)

70. Mahmood, R., J. C. Hines & D. S. Ray: Identification of cis and trans elements involved in the cell cycle regulation of multiple genes in *Crithidia fasciculata*. *Mol Cell Biol*, 19, 6174-82. (1999)

71. Bhandari, D. & P. Saha: mRNA cycling sequence binding protein from *Leishmania donovani* (LdCSBP) is covalently modified by ubiquitination. *FEMS Microbiol Lett*, 273, 206-13 (2007)

72. Gebauer, F. & M. W. Hentze: Molecular mechanisms of translational control. *Nat Rev Mol Cell Biol*, 5, 827-35 (2004)

73. Holcik, M. & N. Sonenberg: Translational control in stress and apoptosis. *Nat Rev Mol Cell Biol*, 6, 318-27 (2005)

74. Kuersten, S. & E. B. Goodwin: The power of the 3' UTR: translational control and development. *Nat Rev Genet*, 4, 626-37 (2003)

75. Akopyants, N. S., R. S. Matlib, E. N. Bukanova, M. R. Smeds, B. H. Brownstein, G. D. Stormo & S. M. Beverley: Expression profiling using random genomic DNA microarrays identifies differentially expressed genes associated with three major developmental stages of the protozoan parasite *Leishmania major*. *Mol Biochem Parasitol*, 136, 71-86 (2004)

76. Cohen-Freue, G., T. R. Holzer, J. D. Forney & W. R. McMaster: Global gene expression in *Leishmania*. *Int J Parasitol*, 37, 1077-86 (2007)

77. Leifso, K., G. Cohen-Freue, N. Dogra, A. Murray & W. R. McMaster: Genomic and proteomic expression analysis of *Leishmania* promastigote and amastigote life stages: the *Leishmania* genome is constitutively expressed. *Mol Biochem Parasitol*, 152, 35-46 (2007)

78. El Fakhry, Y., M. Ouellette & B. Papadopoulou: A proteomic approach to identify developmentally regulated proteins in *Leishmania infantum*. *Proteomics*, 2, 1007-17. (2002)

79. Bente, M., S. Harder, M. Wiesgigl, J. Heukeshoven, C. Gelhaus, E. Krause, J. Clos & I. Bruchhaus: Developmentally induced changes of the proteome in the protozoan parasite *Leishmania donovani. Proteomics*, 3, 1811-29. (2003)

80. McNicoll, F., J. Drummelsmith, M. Muller, E. Madore, N. Boilard, M. Ouellette & B. Papadopoulou: A combined proteomic and transcriptomic approach to the study of stage differentiation in *Leishmania infantum. Proteomics*, 6, 3567-81 (2006)

81. Rosenzweig, D., D. Smith, F. Opperdoes, S. Stern, R. W. Olafson & D. Zilberstein: Retooling *Leishmania* metabolism: from sand fly gut to human macrophage. *Faseb J*, 22, 590-602 (2008)

82. Depledge, D. P., K. J. Evans, A. C. Ivens, N. Aziz, A. Maroof, P. M. Kaye & D. F. Smith: Comparative expression profiling of *Leishmania*: modulation in gene expression between species and in different host genetic backgrounds. *PLoS Negl Trop Dis*, 3, e476 (2009)

83. Soto, M., L. Quijada, C. Alonso & J. M. Requena: Histone synthesis in *Leishmania infantum* is tightly linked to DNA replication by a translational control. *Biochem J*, 346 Pt 1, 99-105. (2000)

84. Soto, M., S. Iborra, L. Quijada, C. Folgueira, C. Alonso & J. M. Requena: Cell-cycle-dependent translation of histone mRNAs is the key control point for regulation of histone biosynthesis in *Leishmania infantum. Biochem J*, 379, 617-25 (2004)

85. Osley, M. A.: The regulation of histone synthesis in the cell cycle. *Annu Rev Biochem*, 60, 827-61 (1991)

86. Stein, G. S., J. L. Stein, A. J. van Wijnen & J. B. Lian: Histone gene transcription: a model for responsiveness to an integrated series of regulatory signals mediating cell cycle control and proliferation/differentiation interrelationships. *J Cell Biochem*, 54, 393-404. (1994)

87. Abanades, D. R., L. Ramirez, S. Iborra, K. Soteriadou, V. M. Gonzalez, P. Bonay, C. Alonso & M. Soto: Key role of the 3' untranslated region in the cell cycle regulated expression of the *Leishmania infantum* histone H2A genes: minor synergistic effect of the 5' untranslated region. *BMC Mol Biol*, 10, 48 (2009)

88. Larreta, R., M. Soto, L. Quijada, C. Folgueira, D. R. Abanades, C. Alonso & J. M. Requena: The expression of HSP83 genes in *Leishmania infantum* is affected by temperature and by stage-differentiation and is regulated at the levels of mRNA stability and translation. *BMC Mol Biol*, 5, 3 (2004)

89. Folgueira, C., L. Quijada, M. Soto, D. R. Abanades, C. Alonso & J. M. Requena: The translational efficiencies of the two *Leishmania infantum* HSP70 mRNAs, differing in their 3'-untranslated regions, are affected by shifts in the temperature of growth through different mechanisms. *J Biol Chem*, 280, 35172-83 (2005)

90. David, M., I. Gabdank, M. Ben-David, A. Zilka, I. Orr, D. Barash & M. Shapira: Preferential translation of Hsp83 in *Leishmania* requires a thermosensitive polypyrimidine-rich element in the 3' UTR and involves scanning of the 5' UTR. *RNA*, 16, 364-74 (2010)

91. Clos, J. & S. Krobitsch: Heat shock as a regular feature of the life cycle of *Leishmania* parasites. *American Zoologist*, 39, 848-856 (1999)

92. Folgueira, C. & J. M. Requena: A postgenomic view of the heat shock proteins in kinetoplastids. *FEMS Microbiol Rev*, 31, 359-77 (2007)

93. Folgueira, C., C. Cañavate, C. Chicharro & J. M. Requena: Genomic organization and expression of the HSP70 locus in New and Old World *Leishmania* species. *Parasitology*, 134, 369-377 (2007)

94. McNicoll, F., M. Muller, S. Cloutier, N. Boilard, A. Rochette, M. Dube & B. Papadopoulou: Distinct 3'-untranslated region elements regulate stage-specific mRNA accumulation and translation in *Leishmania. J Biol Chem*, 280, 35238-46 (2005)

95. Kuhn, D. & M. Wiese: LmxPK4, a mitogen-activated protein kinase kinase homologue of *Leishmania mexicana* with a potential role in parasite differentiation. *Mol Microbiol*, 56, 1169-82 (2005)

96. Besse, F. & A. Ephrussi: Translational control of localized mRNAs: restricting protein synthesis in space and time. *Nat Rev Mol Cell Biol*, 9, 971-80 (2008)

97. Kloc, M., N. R. Zearfoss & L. D. Etkin: Mechanisms of subcellular mRNA localization. *Cell*, 108, 533-44 (2002)

98. Van de Bor, V. & I. Davis: mRNA localisation gets more complex. *Curr Opin Cell Biol*, 16, 300-7 (2004)

99. Lunde, B. M., C. Moore & G. Varani: RNA-binding proteins: modular design for efficient function. *Nat Rev Mol Cell Biol*, 8, 479-90 (2007)

100. De Gaudenzi, J., A. C. Frasch & C. Clayton: RNA-binding domain proteins in Kinetoplastids: a comparative analysis. *Eukaryot Cell*, 4, 2106-14 (2005)

101. Bates, E. J., E. Knuepfer & D. F. Smith: Poly(A)-binding protein I of *Leishmania*: functional analysis and localisation in trypanosomatid parasites. *Nucleic Acids Res*, 28, 1211-20. (2000)

102. D'Orso, I. & A. C. Frasch: TcUBP-1, a developmentally regulated U-rich RNA-binding protein involved in selective mRNA destabilization in trypanosomes. *J Biol Chem*, 276, 34801-9 (2001)

103. De Gaudenzi, J. G., I. D'Orso & A. C. C. Frasch: RNA recognition motif-type RNA-binding proteins in *Trypanosoma cruzi* form a family involved in the interaction with specific transcripts *in vivo*. *J Biol Chem*, 278, 18884-94. (2003)

104. Noe, G., J. G. De Gaudenzi & A. C. Frasch: Functionally related transcripts have common RNA motifs for specific RNA-binding proteins in trypanosomes. *BMC Mol Biol*, 9, 107 (2008)

105. Cassola, A., J. G. De Gaudenzi & A. C. Frasch: Recruitment of mRNAs to cytoplasmic ribonucleoprotein granules in trypanosomes. *Mol Microbiol*, 65, 655-70 (2007)

106. Estevez, A. M.: The RNA-binding protein TbDRBD3 regulates the stability of a specific subset of mRNAs in trypanosomes. *Nucleic Acids Res*, 36, 4573-86 (2008)

107. Walrad, P., A. Paterou, A. Acosta-Serrano & K. R. Matthews: Differential trypanosome surface coat regulation by a CCCH protein that co-associates with procyclin mRNA cis-elements. *PLoS Pathog*, 5, e1000317 (2009)

108. Paterou, A., P. Walrad, P. Craddy, K. Fenn & K. Matthews: Identification and stage-specific association with the translational apparatus of TbZFP3, a CCCH protein that promotes trypanosome life-cycle development. *J Biol Chem*, 281, 39002-13 (2006)

109. Gerber, A. P., D. Herschlag & P. O. Brown: Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. *PLoS Biol*, 2, 342-354 (2004)

110. Wickens, M., D. S. Bernstein, J. Kimble & R. Parker: A PUF family portrait: 3'UTR regulation as a way of life. *Trends Genet*, 18, 150-7. (2002)

111. Spassov, D. S. & R. Jurecic: The PUF family of RNA-binding proteins: does evolutionarily conserved structure equal conserved function? *IUBMB Life*, 55, 359-66 (2003)

112. Caro, F., N. Bercovich, C. Atorrasagasti, M. J. Levin & M. P. Vazquez: *Trypanosoma cruzi*: analysis of the complete PUF RNA-binding protein family. *Exp Parasitol*, 113, 112-24 (2006)

113. Luu, V. D., S. Brems, J. D. Hoheisel, R. Burchmore, D. L. Guilbride & C. Clayton: Functional analysis of *Trypanosoma brucei* PUF1. *Mol Biochem Parasitol*, 150, 340-9 (2006)

114. Folgueira, C., M. Martinez-Bonet & J. M. Requena: The *Leishmania infantum* PUF proteins are targets of the humoral response during visceral leishmaniasis. *BMC Res Notes*, 3, 13 (2010)

115. Hoek, M., T. Zanders & G. A. Cross: *Trypanosoma brucei* expression-site-associated-gene-8 protein interacts with a Pumilio family protein. *Mol Biochem Parasitol*, 120, 269-83 (2002)

116. Dallagiovanna, B., A. Correa, C. M. Probst, F. Holetz, P. Smircich, A. M. de Aguiar, F. Mansur, C. V. da Silva, R. A. Mortara, B. Garat, G. A. Buck, S. Goldenberg & M. A. Krieger: Functional genomic characterization of mRNAs associated with TcPUF6, a pumilio-like protein from *Trypanosoma cruzi*. *J Biol Chem*, 283, 8266-73 (2008)

117. Holetz, F. B., A. Correa, A. R. Avila, C. V. Nakamura, M. A. Krieger & S. Goldenberg: Evidence of P-body-like structures in *Trypanosoma cruzi*. *Biochem Biophys Res Commun*, 356, 1062-7 (2007)

118. Archer, S. K., V. D. Luu, R. A. de Queiroz, S. Brems & C. Clayton: *Trypanosoma brucei* PUF9 regulates mRNAs for proteins involved in replicative processes over the cell cycle. *PLoS Pathog*, 5, e1000565 (2009)

119. Izquierdo, J. M., N. Majos, S. Bonnal, C. Martinez, R. Castelo, R. Guigo, D. Bilbao & J. Valcarcel: Regulation of Fas alternative splicing by antagonistic effects of TIA-1 and PTB on exon definition. *Mol Cell*, 19, 475-84 (2005)

120. Stern, M. Z., S. K. Gupta, M. Salmon-Divon, T. Haham, O. Barda, S. Levi, C. Wachtel, T. W. Nilsen & S. Michaeli: Multiple roles for polypyrimidine tract binding (PTB) proteins in trypanosome RNA metabolism. *Rna*, 15, 648-65 (2009)

121. Jager, A. V., J. G. De Gaudenzi, A. Cassola, I. D'Orso & A. C. Frasch: mRNA maturation by two-step trans-splicing/polyadenylation processing in trypanosomes. *Proc Natl Acad Sci U S A*, 104, 2035-42 (2007)

122. Cuervo, P., G. B. Domont & J. B. De Jesus: Proteomics of trypanosomatids of human medical importance. *J Proteomics*, 73, 845-67 (2010)

123. Rosenzweig, D., D. Smith, P. J. Myler, R. W. Olafson & D. Zilberstein: Post-translational modification of cellular proteins during *Leishmania donovani* differentiation. *Proteomics*, 8, 1843-50 (2008)

124. Morales, M. A., R. Watanabe, M. Dacher, P. Chafey, J. Osorio y Fortea, D. A. Scott, S. M. Beverley, G. Ommen, J. Clos, S. Hem, P. Lenormand, J. C. Rousselle, A. Namane & G. F. Spath: Phosphoproteome dynamics reveal heat-shock protein complexes specific to the *Leishmania donovani* infectious stage. *Proc Natl Acad Sci U S A*, 107, 8381-6 (2010)

125. Weise, F., Y. D. Stierhof, C. Kuhn, M. Wiese & P. Overath: Distribution of GPI-anchored proteins in the protozoan parasite *Leishmania*, based on an improved ultrastructural description using high-pressure frozen cells. *J Cell Sci*, 113 Pt 24, 4587-603. (2000)

126. Macdonald, M. H., C. J. Morrison & W. R. McMaster: Analysis of the active site and activation mechanism of the

Leishmania surface metalloproteinase GP63. *Biochim Biophys Acta*, 1253, 199-207. (1995)

127. McGwire, B. S. & K. P. Chang: Posttranslational regulation of a Leishmania HEXXH metalloprotease (gp63). The effects of site-specific mutagenesis of catalytic, zinc binding, N-glycosylation, and glycosyl phosphatidylinositol addition sites on N-terminal end cleavage, intracellular stability, and extracellular exit. *J Biol Chem*, 271, 7903-9. (1996)

128. McGwire, B. S., W. A. O'Connell, K. P. Chang & D. M. Engman: Extracellular release of the glycosylphosphatidylinositol (GPI)-linked *Leishmania* surface metalloprotease, gp63, is independent of GPI phospholipolysis: implications for parasite virulence. *J Biol Chem*, 277, 8802-9. (2002)

129. Yao, C., J. Luo, P. Storlie, J. E. Donelson & M. E. Wilson: Multiple products of the *Leishmania chagasi* major surface protease (MSP or GP63) gene family. *Mol Biochem Parasitol*, 135, 171-83 (2004)

130. Yao, C., J. Luo, C. Hsiao, J. E. Donelson & M. E. Wilson: Internal and surface subpopulations of the major surface protease (MSP) of *Leishmania chagasi*. *Mol Biochem Parasitol*, 139, 173-83 (2005)

131. Dubessay, P., C. Blaineau, P. Bastien, L. Tasse, J. Van Dijk, L. Crobu & M. Pages: Cell cycle-dependent expression regulation by the proteasome pathway and characterization of the nuclear targeting signal of a *Leishmania major* Kin-13 kinesin. *Mol Microbiol*, 59, 1162-74 (2006)

132. Richard, D., P. Leprohon, J. Drummelsmith & M. Ouellette: Growth phase regulation of the main folate transporter of *Leishmania infantum* and its role in methotrexate resistance. *J Biol Chem*, 279, 54494-501 (2004)

133. Ortiz, D., M. A. Sanchez, S. Pierce, T. Herrmann, N. Kimblin, H. G. Archie Bouwer & S. M. Landfear: Molecular genetic analysis of purine nucleobase transport in *Leishmania major*. *Mol Microbiol*, 64, 1228-43 (2007)

134. Landfear, S. M.: Molecular genetics of nucleoside transporters in *Leishmania* and African trypanosomes. *Biochem Pharmacol*, 62, 149-55. (2001)

135. Hentschel, C. C. & M. L. Birnstiel: The organization and expression of histone gene families. *Cell*, 25, 301-13. (1981)

Abbreviations: ChIP, chromatin immunoprecipitation; DGCs, directional gene clusters; nt, nucleotide or nucleotides; polII, RNA polymerase II; PTMs, post-translational modifications; PTUs, polycistronic transcription units; RBPs, RNA-binding proteins; SSRs, strand-switch regions; UTRs, untranslated regions

Send correspondence to: Jose M. Requena, Centro de Biología Molecular Severo Ochoa, Universidad Autonoma de Madrid, c/ Nicolss Cabrera 1, 28049 Madrid, Spain, Tel: 34-911964617, Fax: 34-911964420, E-mail: jmrequena@cbm.uam.es