RNA-binding proteins and mRNA turnover in trypanosomes

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Trypanosomes, protozoan parasites of the order Kinetoplastida, control gene expression essentially through post-transcriptional mechanisms. Several motifs located mainly in the 3’ untranslated region, such as AU-rich elements (AREs), were recently shown to modulate mRNA half-life, and are able to modify mRNA abundance in vivo through the interaction with specific RNA-binding proteins. Along with the detection of an active exosome, decapping activities and a regulated 3’ to 5’ exonuclease activity stimulated by AREs, these results suggest that modulation of mRNA stability is essential in trypanosomes. These regulatory processes are specific for different developmental stages and thus relevant for allowing trypanosomes to adapt to variable environmental conditions.

The many steps from RNA transcription to protein translation are linked [1]. Among them, the mechanisms that are subject to post-transcriptional regulation include cap addition, splicing and 3’-end processing, nucleocytoplasmic export, mRNA decay and translation. These regulatory pathways are better known in higher eukaryotes than in kinetoplastid parasites (Fig. 1). The lack of transcription initiation control in these kinetoplastid parasites revealed the importance of specific post-transcriptional processes, such as trans-splicing and mRNA stabilization. Recent advances in the study of regulation of nuclear gene expression in trypanosomes are discussed, and the role of RNA-binding proteins that modulate mRNA stability in vivo is compared with the processes taking place in other cell types.

Transcription and RNA processing

Several special features in the regulation of gene expression in kinetoplastid parasites have been identified. (1) No common RNA polymerase II (RNA Pol II) promoters have been found with the exception of the spliced leader (SL) promoter [2]. (2) Few genes, such as those coding the variable surface glycoprotein (VSG) and the procyclic acid repetitive protein (PARP), are transcribed by RNA Pol I [3]. (3) Transcription is polycistronic, and requires efficient and highly progressive transcriptional machinery. (4) The genomic structure and transcription patterns observed in these organisms are unique. Instead of possessing a strict transcriptional control, such as one promoter for each gene, the same region controls several genes in a polycistron. (5) Gene organization is reminiscent of bacterial operons; in particular, the open reading frames (ORFs) are not, in general, interrupted by introns. (6) The distribution and orientation of genes in the genome involves, in several cases, bi-directional promoters and array of genes oriented to both telomeres (reviewed in Ref. [4]).

After transcription, polycistronic pre-mRNA is processed by trans-splicing at the 5’-end and by polyadenylation at the 3’-end, which are two physically coupled and dependent mechanisms [5]. mRNA receive the capped 39 nucleotides (nt) SL sequence at the 5’-end. Once processed, the mature mRNA needs to be exported to the cytoplasm to be efficiently translated or masked until translation is required. Little information is available on the shuttling of ribonucleoprotein complexes (mRNP) through the nuclear pore complex into the cytoplasm. However, in other cell types, proteins are assembled on the unspliced mRNA, a process that is required for splicing, nonsense-mediated decay and transport to the cytoplasm [6].

cis-elements and mRNA half-life

During parasite transmission, adaptation to different environments seems to be achieved by a rapid change in mRNA half-life and translational control, rather than transcriptional activation. Such regulatory activity is due to the interaction between cis elements and trans-acting factors. The cis elements were mainly identified in the 3’ untranslated region (UTR), but also in 5’UTRs and coding regions (see Ref. [4], and references therein). Sequences, such as the AU-rich elements (AREs), were identified in the 3’UTR of the small mucin gene (SMUG) and EP-rich procyclin (EP1) mRNAs in Trypanosoma cruzi and Trypanosoma brucei, respectively [7–9]. These elements were shown to confer selective mRNA destabilization in a stage-specific manner and, at least in T. cruzi, to be recognized by specific trans-acting factors [8,10]. In addition, G-rich elements (GREs) were described in the 3’UTR of SMUG that did not have any structural or sequence identity with other defined elements. GRE and ARE are functionally different, conferring stage-specific mRNA stability and instability, respectively [8].

In Leishmania, a common mechanism of stage-regulated gene expression process mediated by a conserved 3’UTR 450 nt cis element was described [11]. This element is highly conserved among a large number of mRNAs, and is associated with a stage-specific expression pattern in the intracellular amastigote form of the parasite.
RNA-binding proteins

In higher eukaryotic cells, many classes of RNA-binding proteins (RBPs) have been identified, and found to have different cellular functions [6], however, this is not the case for trypanosomes. Few trans-acting factors have been described; some based on sequence identity or the presence of RNA-binding modules (Table 1). First, the poly(A)-binding protein (PABP1) was shown to be present in T. cruzi, T. brucei and Leishmania spp. [12–14]. It has been suggested to be involved in mRNA stability and translation through the interaction with poly(A)-tail in the 3′-end of all mRNAs. Second, two novel proteins (p34 and p37) were shown to interact with 5S ribosomal RNA (rRNA) in T. brucei, suggesting its role in rRNA biogenesis [15]. Both proteins interact with NOPP44/46, the major tyrosine-phosphorylated nucleolar RBPs family in T. brucei [16,17]. Finally, proteins that recognize the 5′-SL RNA sequence, and Ser-Arg-rich (SR-rich) proteins that are similar to RNA-binding factors were identified in T. brucei (Table 1) [18–20]. In T. cruzi, a SR RBP (TcSR) is able to act in cis splicing in a heterologous system [21].

An interesting model to study post-transcriptional regulation and also allelic exclusion is the expression site (ES) loci of T. brucei, a genomic platform and scaffold related with switching mechanisms for VSG expression [22]. A PUF family member, named TbPUF1, was described in T. brucei [23]. The first two members of the PUF protein family analyzed were Drosophila melanogaster PUMILIO and Caenorhabditis elegans FBF [24]. TbPUF1 protein was shown to interact with an ES-associated gene and might regulate the stability of specific ES-derived mRNAs [23]. In eukaryotic cells, members of the family of PUF

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Fig. 1. Comparison of RNA-processing mechanisms in (a) yeast and mammalian cells and (b) trypanosomes. The overall steps (blue boxes) forming part of mRNA maturation processes (after transcription) are shown in both cell types. Molecules forming part of the different RNA-processing mechanisms are indicated. The grey circle in the nucleus of trypanosome cells indicates the presence of a trans-splicing and polyadenylation machinery. See Table 2 for some of the factors acting on each regulatory step. Abbreviations: A, adenylic acid; ARE, AU-rich element; AUBP, AU-rich RNA-binding protein; CBP, cap-binding complex; DCP, decapping complex; eIF, eukaryotic translation initiation factor; EJC, exon-junction complex; hnRNP, heteronuclear ribonucleoprotein; HuR, human RNA-binding protein; In, intron; m7G-cap, tri-methyl-guanosine or CAP structure; mRNA, messenger RNA; NPC, nuclear pore complex; PABP, poly(A)-binding protein; PARN, poly(A)-ribonuclease; RNA Pol II, RNA polymerase II; SL, spliced leader; TcUBP, Trypanosoma cruzi U-rich-binding protein; TBP, TATA-binding protein.

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regulators bind to 3′UTRs and modulate mRNA stability, translation and localization [24].

A single RRM-containing RBP named TcUBP-1, involved in the in vitro destabilization of SMUG mRNAs in a stage-specific manner, has been identified (Fig. 1) [10]. TcUBP-1 is encoded by a single-copy gene, and is a member of the TcUBP family. Five additional members of this family have been cloned recently (Table 1). Preliminary work suggests that they present a single RRM motif, as does TcUBP-1, and acts with different RRM-type RNA-binding proteins [25].

### Table 1. Characteristics of representative RNA-binding proteins in trypanosomes

<table>
<thead>
<tr>
<th>Protein</th>
<th>Organism</th>
<th>Stage of expression</th>
<th>Possible functions</th>
<th>Domain structure</th>
<th>Size (kDa)</th>
<th>Refs</th>
</tr>
</thead>
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<tr>
<td>TcPABP1</td>
<td>T. cruzi</td>
<td>E, T, A</td>
<td>mRNA translation</td>
<td>4-RRM, PABC-domain</td>
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<td>[12–14]</td>
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<td>mRNA translation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TcUBP-1</td>
<td>T. cruzi</td>
<td>E, T, A</td>
<td>mRNA stability</td>
<td>RRM</td>
<td>27</td>
<td>[10]</td>
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<td>E</td>
<td>mRNA stability</td>
<td>RRM</td>
<td>18</td>
<td>[25]</td>
</tr>
<tr>
<td>TcRBP-3</td>
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<td>E</td>
<td>Binding poly(rA, rC, rG) in vitro</td>
<td>RRM</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>TcRBP-4</td>
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<td>16</td>
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</tr>
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<td>T, A</td>
<td>Binding poly(rG, rU) in vitro</td>
<td>RRM</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>TcRBP-6</td>
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<td>Binding poly(rG) in vitro</td>
<td>RRM</td>
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<td>ND</td>
<td>cis-splicing</td>
<td>2-RRM, SR domain</td>
<td>ND</td>
<td>[21]</td>
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<td>NR</td>
<td>PUF repeats</td>
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<td>[23]</td>
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<td>PC, BT</td>
<td>3′-5′ exonuclease exosome subunit</td>
<td>S1 RNA-binding</td>
<td>32.7</td>
<td>[27]</td>
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<td>PC</td>
<td>Exosome subunit</td>
<td>S1 RNA-binding</td>
<td>32</td>
<td>[27]</td>
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<td>RNAse PH</td>
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<td>Binding poly(rU) in vitro</td>
<td>RGG</td>
<td>44/46</td>
<td>[16]</td>
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<td>RRM</td>
<td>34</td>
<td>[15]</td>
</tr>
<tr>
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<td>PC, BT</td>
<td>Binding 5S rRNA</td>
<td>RRM</td>
<td>37</td>
<td>[15]</td>
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<tr>
<td>X81</td>
<td>T. cruzi</td>
<td>E, ND in T, ND in A</td>
<td>Binding stem II of SL RNA</td>
<td>Similar to ScPRP31p</td>
<td>45</td>
<td>[20]</td>
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<td>T. brucei</td>
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<td>2-RRM, SR domain</td>
<td>43</td>
<td>[18]</td>
</tr>
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<td>T5R1IP</td>
<td>T. brucei</td>
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<td>Binding 5′ SL RNA</td>
<td>RNAse, SR-rich</td>
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<td>[19]</td>
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<tr>
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<td>Life cycle regulation</td>
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<td>Life cycle regulation</td>
<td>CCCH</td>
<td>15.7</td>
<td>[30]</td>
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</table>

*This is not a complete list of RNA-binding proteins identified in trypanosomes. Abbreviations: A, amastigotes; BT, bloodstream; E, epimastigotes; ES, expression site; mRNA, messenger RNA; ND, not determined; PABC, poly(A)-binding C-terminal domain; PC, procyclic; RRM, RNA-recognition motif; RGG, Arg-Gly-Gly repeats module; SL, spliced leader; SR, serine–arginine; T, trypanosomes.

Molecular mass estimated from sodium dodecyl sulfate polyacrylamide gel electrophoresis.

*J.G. De Gaudenzi et al. unpublished.

This function has been assigned in splicing assays using yeast and mammalian cells.

RNA–protein interactions

The assembly of mRNP complexes is important to fulfill different cellular functions in a compartmentalized manner. Regarding the regulatory process for mRNA turnover, we have shown that TcUBP-1 interacts directly with another RRM-type RBP, named TcPABP2, in the cytoplasm (Table 1) of the epimastigotes, forming part of a mRNP complex. The poly(A)-binding protein TcPABP1 also interacts with TcUBP-1 in both epimastigote and trypomastigote stages [25]. Because TcUBP-1 is a destabilizing factor [10] and TcUBP-2 is only expressed in epimastigotes [25], we suggest that, in the epimastigote stage, the presence of TcUBP-2 and other yet unidentified factors might stabilize the interaction between TcUBP-1 and TcPABP1 within the mRNP complex. Conversely, this does not occur in the trypomastigote stage, where SMUG mRNAs are destabilized as a result of TcUBP-1 and TcPABP1 direct interaction. Thus, this TcUBP-1-containing mRNP complex might be a clue to study mRNA stabilization/destabilization mechanisms acting in the different stages of the parasite life cycle (Fig. 1). There is no clear evidence for signal transduction pathways governing trypanosome mRNA metabolism yet. Hence, the connection between environmental stimulus and signal transduction to regulate mRNA turnover is a challenge to be solved.

The exosome and mRNA decay mechanisms

mRNA degradation can be achieved by the 5′–3′ and 3′–5′ exonucleolytic pathways. In the 5′–3′ pathway, decapping precedes 5′–3′ mRNA decay, and deadenylation is important for the 3′–5′ cleavage [26]. An exosome, a protein complex with ribonuclease activity, has been recently identified in T. brucei [27], but only eight subunits compose it. Thus, the trypanosome exosome is smaller than that of yeast and mammals (Table 2). Its identification suggests that an early mechanism of mRNA maturation mediated by 3′–5′ exonucleases involved in 3′-end processing, might be similar to, although more primitive than, that found in higher eukaryotic cells. Exosomes from higher eukaryotes and trypanosomes both have a role in 5.8S rRNA processing, but those of higher eukaryotes are also involved in rapid degradation of ARE-containing mRNAs [28]. Therefore, the possibility of regulating the decay of ARE-containing mRNA within its UTRs exists in trypanosomes.

The first description of mRNA-decay processes were described in Ref. [9], showing that mRNAs containing a
poly(A) polymerase; RBPs, RNA-binding proteins; RNA Pol, RNA polymerase; SR, serine–arginine; NXF, nuclear export factor; TcUBP, heteronuclear ribonucleoprotein; HuR, human RNA-binding protein; NES, nuclear export signal; PABP, poly(A) binding protein; PAIP, poly(A)-binding interacting protein; PAP, polyadenylation element-binding protein; CPSF, cleavage and polyadenylation specificity factor; DCP, decapping complex; eIF, eukaryotic translator initiator factor; hnRNP, protein.

[26]; (2) a scavenger activity that generates m7GMP from mediated decay; and (3) a 3′-untranslated region and that is functionally similar to yeast mRNA decapping enzyme (DCP1/DCP2) proteins [26]; (2) a scavenger activity that generates m7GMP from short-capped RNAs and plays a major role in exosome-mediated decay; and (3) a 3′–5′ exonuclease activity stimulated by AREs. This cell free system has defined several steps that probably contribute to regulating mRNA turnover in trypanosomes [29].

26 nt instability element are degraded in a developmentally regulated manner by deadenylation and degradation, using both 5′–3′ and 3′–5′ exonucleolytic pathways. However, it is not clear if the instability element might cause extremely rapid deadenylation, or it might be targeted by an endonuclease. Recently, three protein activities have been identified using cell-free extracts: (1) a decapping activity that removed m7 guanosine diphosphate (GDP) at the 5′-end of the mRNA and that is functionally similar to yeast mRNA decapping enzyme (DCP1/DCP2) proteins [26]; (2) a scavenger activity that generates m7GMP from short-capped RNAs and plays a major role in exosome-mediated decay; and (3) a 3′–5′ exonuclease activity stimulated by AREs. This cell free system has defined several steps that probably contribute to regulating mRNA turnover in trypanosomes [29].

**Perspective**

The number of RBPs described in trypanosomes is growing. However, it is necessary to identify the transcripts that interact with each specific protein, and understand how they are assembled in regulatory pathways. Genomic array analysis of endogenous trypanosome mRNP complexes, combined with proteomics and mass spectrometry-assisted analysis, are excellent tools that will help to reveal: (1) mRNA-binding proteins that are associated with specific RNA populations; (2) if some mRNAs can be found in different mRNP complexes; and (3) if individual mRNP composition might change upon cell differentiation and development. All together, this might allow the identification of novel mechanisms present in trypanosomes, and their relationship with processes acting in different cell types (Table 2). The connection between mRNA stability and signal transduction during parasite development remains unknown. Thus, it is of importance to determine any relationship between physiological function and mRNA turnover rates. Finally, these results will allow us to understand the role of proteins in RNA recognition and metabolism, both important challenges that might be used for the design of non-conventional therapeutic agents against trypanosomiasis. As most pharmacologically active compounds bind proteins, RNA provides untapped opportunities for drug development.

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Toxoplasma gondii, sex and premature rejection
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Adaptive sex ratio theory explains why gametocyte sex ratios are female-biased in many populations of apicomplexan parasites such as Plasmodium and Toxoplasma. Recently, Ferguson has criticized this framework and proposed two alternative explanations – one for vector-borne parasites (e.g. Plasmodium) and one for Toxoplasma. Ferguson raises some interesting issues that certainly deserve more empirical attention. However, it should be pointed out that: (1) there are theoretical and empirical problems for his alternative hypotheses; and (2) existing empirical data support the application of sex ratio theory to these parasites, not its rejection.

Ferguson suggests that natural selection has favoured female-biased sex ratios in Plasmodium and related Haemospororin parasites to promote inefficient fertilization as a form of reproductive constraint [1]. He argues that this is advantageous because it reduces the number of oocysts formed, which avoids compromising vector fitness. Even if there is selection to reduce parasite burdens within mosquitoes (a possibility that remains to be theoretically or empirically demonstrated), there are two problems with Ferguson’s suggestion. First, it would be much easier and less wasteful to constrain oocyst numbers by decreasing the number of gametocytes produced. There is abundant evidence that Plasmodium parasites are able to adjust their rate of gametocytegenesis adaptively in response to environmental conditions [2–4]. Second, reproductive restraint achieved through sex ratio adjustment is not necessarily evolutionarily stable. A population minimizing fertilization success with a female-biased sex ratio could easily be invaded by a less-female-biased mutant. This is because the increased mating success of the mutant would

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