We report the characterization of a *Trypanosoma brucei* TBRGG1, a Mitochondrial Oligo(U)-binding Protein That Co-localizes with an *in Vitro* RNA Editing Activity*

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Luc Vanhamme‡, David Perez-Morga, Christian Marchal, Dave Speijer§, Laurence Lambert‡‡, Maurice Geuskens**, Sylvie Alexandre, Naïma Ismaïli, Ulrich Göringer†, Rob Benne§, and Etienne Pays‡‡

From the Department of Molecular Biology, Free University of Brussels, 67 rue des Chevaux, B1640 Rhode St. Genèse, Belgium, ‡‡Department of Biochemistry, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands, and ‡Gene Centre Munich, Max-Planck Institute for Biochemistry, Am Klopfergut 18, D-82152 Martinsried, Germany

Trypanosomes are primitive eukaryotes whose parasitic life cycle involves the differentiation into several successive adaptive forms in different hosts and environments. The main developmental stages are the bloodstream form in the mammalian host and the procyclic form in the tsetse fly vector. Each of these stages is characterized by a major surface protein, the variant surface glycoprotein in the bloodstream form and procyclin in the procyclic form (1, 2). Another major difference between these forms is the energy metabolism. Bloodstream forms possess an inactive mitochondrion and respire through the catabolism of glucose in specialized organelles termed glycosomes, whereas procyclic forms utilize a fully functional mitochondrion for oxidative phosphorylation, and amino acids probably serve as the major carbon source in *vivo* (3). In the bloodstream, the mitochondrion is reactivated when the trypanosomes differentiate from the proliferative slender form into the quiescent stumpy form through several intermediate stages.

The nuclear genome of these organisms appears to be organized in long polycistronic transcription units and probably contains only a few promoters (4–7). The expression of many genes analyzed so far appears to be stage-specific and reflects the developmental stage of the parasite. Interestingly, genes belonging to the same transcription unit are often differentially stage-regulated, indicating that post-transcriptional processes operating at the levels of RNA maturation, stability, and translation are primarily responsible for controlling cellular differentiation (4–7). The primary polycistronic transcripts of trypanosomes are rapidly processed into mature mRNAs by trans-splicing and polyadenylation. These processing events appear to be coupled, the choice of a polyadenylation site being apparently dictated by the position of the downstream splice site, probably through the scanning of transcripts by a multifactorial complex encompassing both processing activities, which is recruited to the RNA by the binding to a polypyrimidine stretch (7, 8). Several observations indicate that trans-splicing and polyadenylation can be stage-regulated. Alternative splicing of transcripts of the *ESAG6* and *TBA1* genes differs between bloodstream and procyclic forms of the parasite (9, 10). Similar results have been obtained regarding the polyadenylation of transcripts for a high mobility group protein (11). In all these processes, it is believed that RNA-binding proteins play a major role, but presently nothing is known about these proteins.

Mitochondrial transcripts of trypanosomes are also subjected to a developmentally regulated post-transcriptional RNA processing, which involves mechanisms of a nature totally different from that of nuclear genes. These transcripts are rendered functional by a complex editing mechanism involving endonuclease(s), terminal uridyl transferase or uridyl exonuclease, and RNA ligase activities, which leads to the insertion and/or deletion of uridylate residues templated by short polyuridylated RNAs termed guide RNAs, all encoded in the mitochondrial genome (12–17). RNA editing allows the expression of several mitochondrial cryptogenes, which encode subunits of the enzymes of a respiratory-redox chain coupled to phosphorylation of ADP, which is only active in procyclic forms.
Here we report the characterization of an RNA-binding protein specific for poly(U), which selectively accumulates in the mitochondrion when this organelle is active. Alternative processing of the transcripts and stage-regulated protein modification suggest that complex controls regulate the expression of TBRGG1. It is likely that this protein is involved in mitochondrial RNA processing, either in rRNA synthesis and maturation or in RNA editing.

**EXPERIMENTAL PROCEDURES**

*Trypanosomes*

*Trypanosoma brucei* pleomorphic bloodstream forms were from the AnTat 1.1 clone. Procyclic forms were derived from these cells by *in vitro* cultivation (18).

**DNA Cloning and Analysis**

A cDNA library was constructed in λgt10 from poly(A)^+ mRNA isolated from bloodstream forms using the Amersham Pharmacia Bio-tech cDNA synthesis and cloning kit according to the instructions of the manufacturer. For *in vitro* transcription and translation, the entire 2.9-kb ORF containing the first 19 nucleotides of the ORF1 contain both ORF1 and TBRGG1. The most 5′-termina19 nucleotides are from the 3′-end of the spliced leader sequence (mini-exon). The two major boxes contain the largest open reading frames of the cDNA. The processing sites in other transcripts from the same region are indicated (black arrowhead, splice acceptor site; open arrowhead, polyadenylation site) (see Fig. 4 for details).

**Fig. 1.** Nucleotide sequence of a full-sized cDNA containing both ORF1 and TBRGG1. The most 5′-terminal 19 nucleotides are from the 3′-end of the spliced leader sequence (mini-exon). The two major boxes contain the largest open reading frames of the cDNA. The processing sites in other transcripts from the same region are indicated (black arrowhead, splice acceptor site; open arrowhead, polyadenylation site) (see Fig. 4 for details).
Production of Specific Antibodies

The TBRGG1 protein and a polypeptide of 238 amino acids (amino acids 30 to 268) containing the RGG repeats (RGG peptide) were expressed in *E. coli* from pGEX2.9 and pGEXRGG. The two GST fusion proteins were purified in large amounts using the Bio-Rad Cell Prep SDS-PAGE apparatus. The fractions containing the purified recombinant proteins were applied on a column of AG11A8 resin to remove the SDS. The RGG peptide was cleaved from the GST moiety using thrombin and repurified using the same procedure. The TBRGG1-GST fusion and the RGG peptide were then used to immunize rabbits and chickens. Chicken egg yolks delipidified with chloroform as well as rabbit antiserum were used for subsequent analyses.

In Vitro Synthesis of TBRGG1 Polypeptides

The TBRGG1 protein and the RGG peptide were synthesized in reticulocyte lysate in the presence of 35S-labeled methionine from pGEM2.9 and pGEMRGG, respectively, using the Promega in vitro transcription and translation systems.

DNA and RNA Binding Assays

2 μl of reticulocyte lysate containing the labeled protein were mixed with 25 μl of agarose beads bound to oligoribonucleotides (poly(A), poly(C), poly(G), and poly(U), purchased from Amersham or Sigma) or single-stranded salmon sperm DNA (purchased from Life Technologies, Inc.), in 0.5 ml of binding buffer (2.5 mM MgCl₂, 0.5% Triton X-100, 10 mM Tris-HCl, pH 7.4, with 250, 500, 750, or 1000 mM NaCl). The binding was allowed to proceed for 10 min at 4 °C with mild agitation. The beads were then pelleted and subjected to 6 washes with 1 ml of binding buffer. Sample buffer was added to the beads, followed by boiling for 5 min and SDS-PAGE analysis. After electrophoresis, the gel was subjected to fluorography, dried, and exposed. UV cross-linking assays were conducted as described previously (19).

Subcellular Fractionation Procedures

**Renografin Gradient**—Mitochondrial vesicle isolation was performed in a 20–35% renografin gradient, essentially as described (20). Renografin gradients were divided into 14 fractions of 2 ml. Before Western blotting, 0.02% laurylmaltoside was added to the fractions followed by dialysis against 5 mM EDTA, 25 mM Tris, pH 7.6. Proteins were precipitated with 10% trichloroacetic acid and resuspended in SDS-PAGE layer mix at a concentration of approximately 3 μg/ml.

**Glycerol Gradient**—Procyclic *T. brucei* IsTaR 1 (21) was grown at 27 °C in SDM-79 medium supplemented with 10% (v/v) heat-inactivated bovine fetal calf serum (22). The preparation of mitochondrial vesicles followed the protocol of Harris et al. (23). Detergent lysates of the vesicle preparations were prepared as described by Göringer et al. (24) using 1% (v/v) Triton X-100 (75-fold critical micellar concentration)
for the solubilization. Protein concentrations were determined according to Bradford (25). Mitochondrial lysates (10 mg) in 20 mM Hepes, pH 8.3, 30 mM KCl, 10 mM magnesium acetate, 5 mM CaCl₂, 0.5 mM dithiothreitol, 1% (v/v) Triton X-100, 1 mM phenylmethlysulfonyl fluoride, 1 μg/ml leupeptin, and 10 μg/ml bovine trypsin inhibitor were fractionated by density centrifugation in linear 10–35% (v/v) glycerol gradients (26). Centrifugation was performed in a Beckman SW 41 rotor at 38,000 rpm for 5 h at 4 °C. Twelve 1-ml fractions were collected from the top of the gradient. The fractions were tested for their in vitro RNA editing activity as described by Seiwert et al. (19).

Affinity Purification of anti-TBRGG1 Antibodies and Immunoblotting

Affinity purification of the antibodies was performed as described (27). Immobilized full-length GST-TBRGG1 fusion protein was used as the antigen. For immunoblotting, protein extracts were separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% (w/v) bovine serum albumin in phosphate-buffered saline and probed with the antibodies.

Western Blotting

The protein samples were run in 10% Laemmli SDS-PAGE. Western blotting was performed by the semi-dry method for 2 h limited at 250 mA onto nitrocellulose. This was followed by standard detection procedures. The antisera were detected using rabbit anti-TBRGG1, a rabbit anti-glucosephosphate isomerase raised against glucosephosphate isomerase of *T. brucei* b, and a rabbit anti-glucosephosphate isomerase raised against glucosephosphate isomerase of *T. brucei*, which in procylic forms is 50% glycosomal and 50% cytoplasmic (a gift of Prof. F. R. Opperdos, Institute for Cellular Pathology, Brussels).

Immunofluorescence

Trypanosomes were fixed in suspension (1.75% paraformaldehyde, 0.25% glutaraldehyde), permeabilized in 0.1% Triton X-100, and processed for indirect immunofluorescence as described previously (28).

FIG. 4. Steady-state transcripts of the TBRGG1 locus. The top panel shows a Northern blot analysis of 10 μg of poly(A)⁺ RNA from bloodstream (b) and procyclic (p) forms of *T. brucei* hybridized under stringent conditions with the cDNA probes shown under the map. The control panel at the right shows the hybridization of a filter from the same Northern series with a *T. brucei* actin probe (act). The actin mRNA is 1.6 kb long and is present in similar amounts in both forms of the parasite (34), whereas the 0.8-kb band present in lane p only is a residual signal from a previous hybridization of this filter with a procyclin probe (pro). In panel 3, the dot partially overlapping with lane b (bottom) is not a RNA band. (A), polyadenylation sites.

FIG. 5. The TBRGG1 protein is present in two to three forms depending on the developmental stage of the parasite. Western blots of 50 μg of total protein extracts from procyclic (p) and bloodstream long slender (b) forms of *T. brucei* were incubated with immune (i) or pre-immune (pi) anti-TBRGG1 antibodies. The arrow points to the procyclic-specific band.

Chicken anti-TBRGG1 was used at a 1:50 dilution, and the fluorescein isothiocyanate-coupled anti-chicken antibody (Sigma) was diluted 320-fold. Images were taken on a Zeiss Axioscop microscope coupled to a CCD camera and processed by a ISIS 3 software.

Electron Microscopy Immunocytochemistry

Pleomorphic bloodstream forms and a purified mitochondrial fraction from procyclic culture forms were fixed and embedded in Lowicryl K4M (Sigma) diluted 320-fold. Images were taken on a Zeiss Axioscop microscope coupled to a CCD camera and processed by a ISIS 3 software.

RESULTS

Cloning of a *T. brucei* Gene for a RNA-binding Protein (TBRGG1)—The cloning of the TBRGG1 gene was initially designed to characterize the trypanosome homologue of nucleolin from higher eukaryotes. Nucleolin is an RNA-binding protein involved in the synthesis and processing of tRNAs (30). We were interested in the identification of a trypanosome homologue of this protein because the transcription of the variant surface glycoprotein and *procyclin* genes appears to be mediated by polymerase I (31), and RNA-binding proteins are likely to represent key control elements for the expression of these genes (2, 4–7). Therefore, we used a probe from hamster nucleolin to screen a *T. brucei* bloodstream form cDNA library cloned in a bacteriophage vector. Several positive clones were obtained, which were found to contain the same sequence, at least partially. The nucleotide sequence of the largest cDNA is shown in Fig. 1. This cDNA (3.4 kb) was full-sized, since at the 5' extremity it contained the end of the common spliced leader (termed mini-exon), and it was polyadenylated at the 3' extremity. Interestingly, this cDNA was found to contain two successive ORFs. The largest one encoded a putative 75-kDa protein showing five typical repeated motifs found in a class of RNA-binding proteins termed RGG, which consist of closely spaced RGG tripeptides interspersed with phenylalanine and/or aromatic amino acids (32) (Fig. 2A and B); hence the trypanosome gene was termed TBRGG1, for *T. brucei* RGG gene 1. The region of the RGG repeats is the only one showing significant homology with hamster nucleolin (30) and was probably responsible for the selection of these clones. The first ORF encoded a putative polypeptide of about 15 kDa, showing no significant homology with sequences from data bases.

Different fragments from the cDNA were used as probes to analyze the nuclear genes by hybridization. As shown in Fig. 3, the different probes confirmed the arrangement of the two successive ORFs. Probe 1, which is specific to ORF1, hybridized to the same 2.2-kb SacI fragment as probe 2, which is specific to the beginning of ORF2 (Fig. 2A and B).

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the map, the same 1.6-kb PstI fragment was recognized by probes 1 and 2, whereas KpnI allowed an identification of the specific fragments hybridizing to each of the two probes. This analysis showed that although the ORF1 region is unique, a few sequences distantly related to the region encompassing TBRGG1 exist elsewhere in the genome (see, for instance, the weakly hybridizing bands at the top of the Ss + S and K lanes in panel 2 or the upper KpnI fragment in panel 3).

Alternative Splicing and Polyadenylation of TBRGG1 Transcripts—To evaluate the significance of the existence of two ORFs in the TBRGG1 cDNA, we used probes specific to either of these ORFs to screen the cDNA library and analyze the pattern of steady-state transcripts. The diagrams at the bottom of Fig. 4 show the structure of different full-sized cDNAs ob-

FIG. 6. Sub-cellular localization of TBRGG1, as determined by immunofluorescence in bloodstream slender forms (3 days of infection) (SLD), short stumpy forms (6 days of infection) (SS), and procyclic forms (P). TBRGG1 was detected using a fluorescein isothiocyanate-coupled anti-chicken antibody after incubation with an anti-TBRGG1 chicken antibody. DAPI was used to stain and localize the kinetoplast (arrowhead) and the nucleus (arrow).

FIG. 7. Localization of TBRGG1, as determined by electron microscopy on sections of mitochondrial vesicles isolated from procyclic forms (panel a) and on cell sections of intermediate bloodstream forms (panel b). Ultrathin sections of Lowicryl-embedded material were incubated first with anti-TBRGG1 antibodies, then with a gold-conjugated secondary antibody. k, kinetoplast; m, mitochondrion; N, nucleus; bar, 1 μm. Incubation with the pre-immune serum led to a weak labeling without any particular localization (not shown).
T. brucei Mitochondrial Oligo(U)-binding Protein

Stage-regulated Modification of the Protein—The entire TBRGG1 gene and the region of the RGG repeats were cloned in an E. coli expression vector, and the recombinant proteins were purified by SDS-PAGE from cells transfected with the appropriate vectors. Antibodies were raised against these polypeptides in rabbits and chickens. Western blot analyses were performed using these antibodies, and similar results were obtained irrespective of the nature of the immunogen or the source of antibodies. As shown in Fig. 5, the TBRGG1 protein was present in both bloodstream and procyclic forms as a doublet or triplet of bands, depending on the antibody and cell extract, and migrated with the relative molecular mass predicted from the nucleotide sequence of the cDNA (about 75 kDa). With both antibodies, the upper band was more visible in the procyclic form (arrow). As the TBRGG1 mRNA appears to be unique (as above), the multiplicity of protein bands may be explained by either modification or proteolysis or by cross-detection of related proteins.

We attempted to determine whether TBRGG1 was modified by phosphorylation (data not shown). First, TBRGG1 was immunoprecipitated from trypanosomes grown in the presence of 32P-orthophosphate and analyzed by SDS-PAGE. No labeling was observed although TBRGG1 was readily immunoprecipitable in control experiments. Second, treatment with phosphatases did not interfere with the pattern of migration in SDS-PAGE.

Since both RGG proteins contain the modified amino acid N\(^{G}\)G\(^{N}\)-dimethylarginine (35), we evaluated the possible involvement of this modification in the observed pattern of bands. The methylation of arginines is inhibited by adenosine dihydro (35). However, after incubation of both bloodstream and procyclic forms for three generation times (respectively, 24 and 50 h) in the presence of sub-toxic concentrations of this compound (10 \(\mu\)M), no change of the electrophoretic pattern was observed (not shown).

It is worth noting that the electrophoretic mobility of the upper band corresponded to that of the in vitro translation product of the TBRGG1 mRNA (data not shown). Therefore, it is possible that the lower bands arise by proteolytic cleavage. In support of this hypothesis, we observed that a second band can appear from the in vitro synthesized protein after prolonged storage.

Subcellular Localization—The use of the chicken anti-TBRGG1 antibodies allowed the demonstration that TBRGG1 is mitochondrial in intermediate and stumpy bloodstream forms. A preferential accumulation of the protein in the mitochondrion of these developmental forms is clearly demonstrated by immunofluorescence (Fig. 6, SS) and electron microscopy (Fig. 7, panel b). In bloodstream slender forms, this preferential localization was not observed (Fig. 6, SLD). In procyclic forms, the protein also appeared to be present in the mitochondrion (Fig. 6, P).

The mitochondrial localization in procyclic forms was verified by renografin (floating density) gradient analysis. Renografin gradients are routinely used to purify trypansomal mitochondrial vesicles (see Ref. 20), which are easily identifiable because of the presence of specific cytochromes. Fig. 8A shows a Western blot comparing equal amounts of total and mitochondrial proteins from procyclic forms probed with both anti-TBRGG1 and anti-mitochondrial ATPase antibodies. The bands, respectively, detected with each of these antibodies alone are indicated by the arrows. The TBRGG1 signal in the mitochondrial fraction was about 10 times stronger than that obtained with the total protein fraction, which is similar to the enrichment observed for the mitochondrial ATPase, providing further evidence for the mitochondrial location of the protein. To check that this result was not due to contamination of the mitochondrial fraction with the glycosomal fraction, the complete renografin gradient was tested in Western blotting assays with different antibodies. As can be seen in Fig. 8B, the TBRGG1 protein colocalized with the mitochondrial ATPase and not with glycosomal glucosephosphate isomerase. The presence of the TBRGG1 protein in the mitochondrial vesicles was directly observed by electron microscopy. Fig. 7a allowed the verification of the purity of the mitochondrial fraction and showed that the labeling is associated with fuzzy material inside the vesicles.

Taken together, these results indicated that, at least at the developmental stages where the mitochondrion is active, TBRGG1 is present in the mitochondrion. Interestingly, the amino acid sequence at the N terminus of TBRGG1 predicted an amphipatic helix compatible with a targeting signal for mitochondrial import (Fig. 2C) (33).

Selective Binding to Poly(U)—The amino acid sequence of the TBRGG1 protein strongly suggested a RNA binding capacity. To check this hypothesis, the protein was synthesized in a...
incubated with the indicated oligonucleotides bound to agarose beads in the presence of increasing concentrations of NaCl (250, 500, 750, and 1000 single-stranded DNA (Fig. 9).

Electrophoretic analysis of the proteins attached to the beads indicated that at low concentrations of salt, TBRGG1 binds to all oligoribonucleotides except poly(C) and does not bind to single-stranded DNA (Fig. 9A). The binding to poly(U) and poly(A), but not to poly(G), was not affected by concentrations of NaCl up to 1 M, showing that TBRGG1 has a preferential affinity for these two oligoribonucleotides. However, the stronger binding to poly(U) clearly indicated that TBRGG1 is a oligo(U)-binding protein.

To evaluate the involvement of the RGG domain in RNA binding, a fragment of 238 amino acids containing the RGG repeats of TBRGG1 was subjected to the same assays, giving similar results as those obtained with the entire protein (Fig. 9B). Therefore, the RGG domain of TBRGG1 accounts for the binding to poly(U).

TBRGG1 Is Most Likely the 83-kDa Oligo(U)-binding Protein, One of the Two Major Guide RNA-binding Proteins of T. brucei—The RGG domain of TBRGG1 and a mitochondrial lysate of T. brucei were incubated with 32P-radiolabeled oligo(U), then cross-linked by UV irradiation under conditions previously described (30). Western blots of these labeled materials were probed with the anti-TBRGG1 antibodies and autoradiographed to visualize the bound oligo(U). As expected, the RGG domain was associated to a strong oligo(U) labeling (Fig. 10, lane 2 in panels A and B). The TBRGG1 protein present in the mitochondrial vesicles was found to precisely comigrate with a major oligo(U)-binding protein with an apparent size of 83 kDa under these conditions, which was described previously as the largest T. brucei protein cross-linkable to synthetic guide RNAs provided they are equipped with a U-tail and to oligo(U) (19) (Fig. 10, lane 1 in panels A and B). This last result was not related to the relative abundance of proteins in the mitochondrial extract, since the labeling did not coincide with a major band as seen after silver staining (not shown). Mixing the RGG domain with the mitochondrial lysate led to a decrease in the intensity of the 83-kDa band, indicating that the RGG domain of TBRGG1 can compete with the endogenous 83-kDa protein for limiting amounts of oligo(U) (data not shown).

TBRGG1 Co-localizes with an in Vitro RNA Editing Activity—The mitochondrial localization as well as the oligo(U)-binding properties of TBRGG1 tempted us to test whether the protein is potentially involved in the RNA editing process. The reaction is likely performed within a highly molecular mass ribonucleoprotein complex (26, 36, 37), which probably involves several protein components in addition to guide RNAs and pre-edited mRNAs. A potential participation of TBRGG1 in this process would predict that some or even the entire mitochondrial TBRGG1 population might co-localize with an in vitro RNA editing activity as a sedimentation analysis. To test this hypothesis, we isolated mitochondrial vesicles from procyclic trypanosomes (23). The vesicles were lysed (24), and the lysates were separated in glycerol density gradients and fractionated (26). Aliquots of the different fractions were separated by SDS-PAGE and analyzed by immunoblotting using affinity-purified anti-TBRGG1 antibody (Fig. 11A). The majority of the protein was detected in fractions 5–9 equivalent to an apparent S-value range of 35–40 S. Quantitating the specific abundance of TBRGG1 in the different fractions resulted in a distribution as shown in Fig. 11B. Fractions 6–8 represented the peak of the separation and contained approximately 75% of the total amount of TBRGG1. Thus, the protein showed a sedimentation behavior suggestive of being associated with or assembled into high molecular mass complexes.

To analyze in vitro RNA editing in the different gradient fractions, we chose to monitor a gRNA-dependent U-deletion activity (13). Educt in the reaction is a synthetic, radiolabeled pre-mRNA derived from the first editing site of the T. brucei ATPase 6 (A6) mRNA. This molecule is edited by the addition of mitochondrial protein extracts and a specific gRNA molecule that directs the deletion of exactly 4 uridylate residues. Typically, the reaction results in the conversion of approximately ±10% educt RNA into the editing product. In addition to monitoring editing product formation, the assay resolves a 3' pre-mRNA cleavage intermediate as well as chimeric gRNA/mRNA side products (13). Uridylate deletion activity was assayed in the first 10 fractions from the top of the gradient. The activity peaked in fractions 6 to 8 (Fig. 11C) and, thus, precisely overlapped with the gradient distribution of TBRGG1 protein.

DISCUSSION

The TBRGG1 protein is the second RNA-binding protein of the RGG type described in T. brucei. The first one, termed Nopp44/46 (38), is exclusively nuclear and, thus, is probably involved in nuclear rRNA synthesis and/or processing. Interestingly, Nopp44/46, like TBRGG1, preferentially binds to poly(U). As in both cases, the RGG domain was found to be entirely responsible for RNA binding; the selective affinity for poly(U) in the two proteins is due to the RGG repeats only.

The transcription and processing pattern of TBRGG1 is probably the most complex reported to date in T. brucei. It
provides the first example of steady-state accumulation of dicistronic transcripts and involves alternative splicing and polyadenylation of the individual mRNAs for each of the two ORFs. The location of the splice and polyadenylation sites of these mRNAs indicated that their maturation is mutually exclusive. This arrangement opens the way for mutually exclusive control of the expression of ORF1 and TBRGG1. However, in the major developmental stages analyzed, bloodstream and culture procyclic forms, both mRNAs and the polypeptides they encode were produced in relatively large amounts. As both trans-splicing and polyadenylation can exhibit stage specificity (9–11), is possible that an exclusive accumulation of either ORF1 or TBRGG1 occurs at a minor stage of the parasite life cycle or depends on particular environmental conditions. This issue could be clarified with the determination of the function of the two proteins.

In addition to the complexity of transcript processing, the TBRGG1 protein was found to exist in several forms, one of which is preferentially found in the procyclic stage of the parasite life cycle. We were unable to ascribe this pattern to differential protein phosphorylation or arginine methylation. Proteolytic cleavage might be responsible, although this remains to be proven. Finally, we cannot exclude cross-detection of other proteins of the RGG type, although one of these, Nopp44/46 (44–46 kDa) (38), is clearly not recognized by the anti-TBRGG1 antibodies. Interestingly, in the Nopp44/46 case also, two to three bands were detected (38). The heterogeneity of TBRGG1 does not seem to be required for sub-cellular targeting, since both major forms appeared to be present in the mitochondrion, as demonstrated by their co-sedimentation all along the renografin gradient.

The role of TBRGG1 is obviously related to the function of the mitochondrion, since this protein is targeted to this organelle as soon as it is reactivated in intermediate bloodstream forms, and it is found in the active mitochondrion of the procyclic form. In a sedimentation analysis of mitochondrial lysates, we were not able to identify free TBRGG1 protein. Instead, the entire TBRGG1 population we found in a high molecular mass region of the glycerol gradient. Based on the nonstringent lysis conditions of the mitochondrial vesicles, we feel it is unlikely that this represents high molecular mass aggregates of TBRGG1. In contrast, we favor the interpretation that the protein is assembled into or associated with mitochondrial complexes of a high structural complexity. Intriguingly, the same gradient fractions that contained TBRGG1 protein also exhibited an in vitro RNA editing activity. Although a co-localization per se is not a conclusive argument for a functional relationship, several characteristics of TBRGG1 support such a participation. First, RNA editing is likely mediated by a complex ribonucleoprotein complex (26, 36, 37). This complex contains pre-edited mRNAs and gRNAs that in a transient form. In a sedimentation analysis of mitochondrial lysates of T. brucei (lanes 1) or the RGG domain of TBRGG1 fused to GST (lanes 2) were incubated with radiolabeled oligo(U) and UV cross-linked as described in Leegwater et al. (19). Western blots of the labeled extracts were probed with anti-TBRGG1 antibodies (panel A) and autoradiographed (panel B). The proteins of 83, 64, 55, and 42 kDa have previously been identified as oligo(U)-binding proteins in the T. brucei mitochondrial lysate (19).
protein components of the editing ribonucleoprotein must contain recognition motifs for the RNA moieties of the complex, such as the RGG box in TBRGG1. Second, the preferential binding to poly(U) suggests an interaction with gRNAs that has been shown to contain 3′ oligo(U) extensions. In accordance with this suggestion, in *T. brucei*, U-binding proteins are found in guide RNA-containing particles in nondenaturing gels, and some of them co-sediment with guide RNAs in glycerol gradients (36, 39). Among the oligo(U)-binding proteins identified by some of them co-sediment with guide RNAs in glycerol gradients, T. *brucei* TBRGG1 has drawn our attention to the putative mitochondrial import sequence of the hamster nucleolin probe and C. Clayton (Heidelberg) for having binding to poly(U) suggests an interaction with gRNAs that has such as the RGG box in TBRGG1. Second, the preferential binding of TBRGG1 to poly(U) would also be compatible with a role in this process.

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