

Identification of novel components of *Trypanosoma brucei* editosomes

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ABSTRACT

The editosome is a multiprotein complex that catalyzes the insertion and deletion of uridylates that occurs during RNA editing in trypanosomatids. We report the identification of nine novel editosome proteins in *Trypanosoma brucei*. They were identified by mass spectrometric analysis of functional editosomes that were purified by serial ion exchange/gel permeation chromatography, immunoaffinity chromatography specific to the TbMP63 editosome protein, or tandem affinity purification based on a tagged RNA editing ligase. The newly identified proteins have ribonuclease and/or RNA binding motifs suggesting nuclease function for at least some of these. Five of the proteins are interrelated, as are two others, and one is related to four previously identified editosome proteins. The implications of these findings are discussed.

Keywords: RNA editing; protein; mass spectrometry; chromatography; TAP-tag; ribonuclease

INTRODUCTION

Uridylate (U) insertion/deletion editing, which appears to be unique to trypanosomatids, processes most mitochondrial pre-mRNAs to produce mature mRNAs (see Kable et al. 1997; Estevez and Simpson 1999; Stuart et al. 2000; Madison-Antenucci et al. 2002; Stuart and Panigrahi 2002). The editing process is performed by the editosome, a multiprotein complex, which catalyzes the series of coordinated enzymatic steps that result in edited RNA. Small (~60 nt) mitochondrial guide RNAs (gRNAs) specify the sites of editing and the number of U insertions and deletions. The first enzymatic step in the editing process is gRNA-directed endonucleolytic cleavage of the pre-mRNA at the editing site. Subsequently, U's are added to the 3' terminus of the 5' cleavage product by 3' Terminal Uridyl Transferase (TUTase) for insertion or are removed by 3' exo-uridylylase (exo-Uase) for deletion. The U addition or deletion step is followed by ligation of 5' and 3' cleavage fragments by RNA ligase. Each gRNA contains sequence information for the editing of multiple sites within a block of 25–35 nt in the mRNA. Most mRNAs require multiple gRNAs to direct the editing of multiple blocks to fully edit the mRNA.

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There has been recent progress in characterizing the editosome. The association of editing with a complex was implied by the findings that activities expected for editing sediment at 20–40S as do gRNAs and pre-mRNAs (Pollard et al. 1992; Corell et al. 1996) and two RNA ligases that were identified by adenylation and deadenylation (Sabatini and Hajduk 1995). SDS-PAGE analysis of editosomes purified by various biochemical methods revealed between 7 and more than 20 major protein bands depending on the procedure (Rusché et al. 1997; Madison-Antenucci et al. 1998; Panigrahi et al. 2001a). The genes for the two RNA Editing Ligases (REL) 1 and 2, formerly TbMP52 and TbMP48, respectively, were identified by mass spectrometry of the purified proteins (Panigrahi et al. 2001a) and shown to encode RNA ligases (McManus et al. 2001; Rusché et al. 2001; Schnauffer et al. 2001) and correspond to bands IV and V (Rusché et al. 1997). TbREL1 was shown by gene inactivation studies to be essential for editing and for viability of the parasite (Schnauffer et al. 2001) and similar studies indicated that TbREL2 is not essential for editing or cell survival (Drozd et al. 2002). Genes for four other editosome proteins, TbMP18, TbMP42, TbMP63, and TbMP81, were also identified by mass spectrometry of purified editosomes (Panigrahi et al. 2001b). These proteins share sequence conservation among themselves to a certain extent and the largest three have zinc fingers. The two largest of these proteins are essential for editing, as inactivation

of expression of the TbMP63 (band III; Huang et al. 2002) or TbMP81 (Drozd et al. 2002) gene block editing and result in the loss of the TbREL1 and TbREL2, respectively. A DEAD box helicase, mHEL61p, has a role in editing, as null mutants of *Trypanosoma brucei* have reduced edited mRNAs in vivo (Missel et al. 1997). A mitochondrial 3' TUTase gene was also found to be essential for editing in *T. brucei* by inhibition of its expression (Aphasizhev et al. 2002).

In addition, several RNA-binding proteins, gBP21 (Koller et al. 1997) and the related gBP25 (Blom et al. 2001), as well as REAP1 (Madison-Antenucci et al. 1998), TBRGG1 (Vanhamme et al. 1998), and RBP16 (Hayman and Read 1999), may have roles in RNA editing. At present, it is unclear if or to what extent these proteins are associated with the editosome. The roles of these proteins may be in gRNA processing, annealing of gRNA and mRNA (Muller et al. 2001), and/or transport of RNAs to the editosome (Madison-Antenucci and Hajduk 2001).

This study describes the identification of nine additional editosome proteins, all of which are stably associated with the ~20S editosome. These proteins are novel but have sequence characteristics suggesting roles in RNA interaction and processing. The editosome proteins identified to date have varying degrees of sequence similarities, which reveal that they occur as pairs and sets of related proteins.

RESULTS

Editosomes were purified by three different approaches to determine their protein content. They were purified from mitochondrial lysates by sequential column chromatography as previously described (Panigrahi et al. 2001a), from glycerol gradient-fractionated mitochondrial lysates by affinity purification using a MAb specific for editosome protein TbMP63 (Panigrahi et al. 2001b), and from total cell lysates of transgenic trypanosomes by the tandem affinity purification (TAP) procedure (Rigaut et al. 1999). Trypanosomes that express TbREL1 with a C-terminal TAP-tag under the control of a tetracycline-inducible promoter were prepared as described in Materials and Methods. The TAP-tagged protein was efficiently expressed in vivo and glycerol gradient analysis showed incorporation into ~20S complexes (results not shown).

The TAP-tagged complexes underwent a first affinity chromatography with an IgG column, which binds the protein A segment of the TAP-tag, and were eluted by cleavage with TEV protease. They then underwent a second affinity chromatography with a calmodulin column, which binds the calmodulin-binding protein (CBP) segment of the TAP-tag, and were eluted using EGTA. Editosomes purified by these three methods all contained the four proteins for which we have MAbs, TbMP81, TbMP63, TbREL1 (TAP-tagged in one case), and TbMP42, as shown by Western analysis (Fig. 1A), and a fifth protein TbREL2, along with TbREL1, was identified by adenylation (Fig. 1B). The editosomes purified by these three methods were all functional in full round in vitro deletion editing (Fig. 1C). Hence, all three methods resulted in functional editosomes.

The proteins in the purified editosomes and their corresponding genes were identified by a combination of liquid chromatography tandem mass spectrometry (LC-MS/MS) and DNA sequence database analysis as described

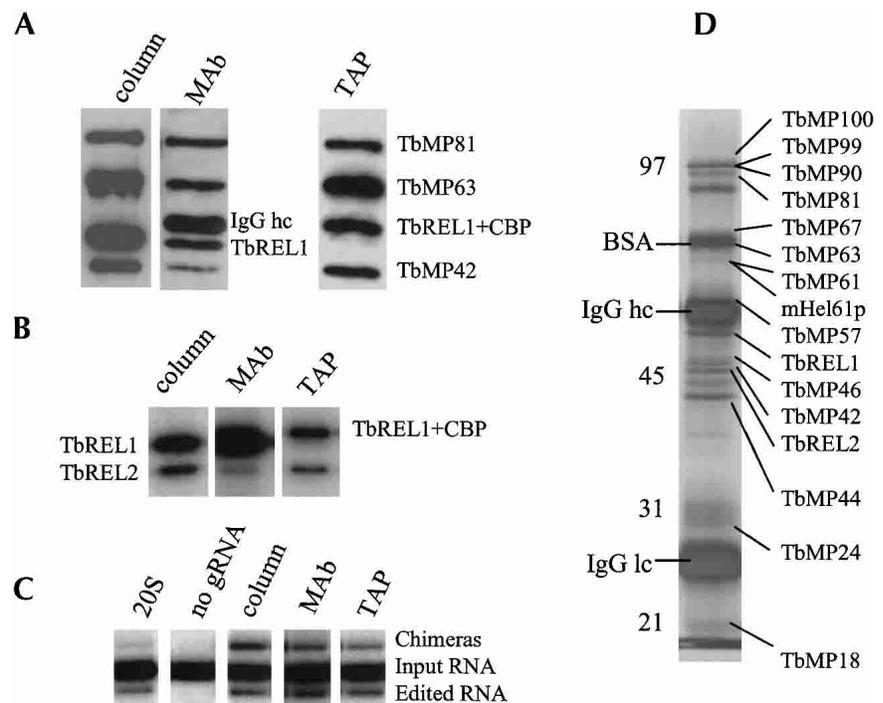


FIGURE 1. Isolation of *T. brucei* editosomes. (A) Western analysis of the complexes purified by column chromatography (sequential ion-exchange and gel filtration), MAb affinity, and the TAP method showing the presence of editosome proteins TbMP81, TbMP63, TbREL1, and TbMP42. IgG heavy chain (hc) and tagged TbREL1 are indicated. (B) Adenylation of editosome preparations. The adenylated TbREL1, TbREL2, and tagged TbREL1 are indicated. (C) In vitro deletion RNA editing assays using the purified editosomes. Mitochondrial 20S fraction served as a positive control; no gRNA indicates the negative control. Input pre-mRNA, resulting edited RNA, and chimeras resulting from ligation of cleaved 3' pre-mRNA to gRNA are indicated. (D) Silver-stained SDS-PAGE protein profile of MAb affinity-purified complexes. Proteins identified by LC-MS/MS are indicated, although some were not identified as discussed in the text. Size standards, bovine serum albumin (BSA) used as a blocking agent, and IgG heavy (hc) and light (lc) chains of the MAb are indicated. The relative migration of some proteins differs from that predicted from the gene sequence and some proteins comigrate.

(Panigrahi et al. 2001b). The editosome proteins were designated TbMP \underline{n} (*Trypanosoma brucei* Mitochondrial Protein with \underline{n} indicating the preprocessed molecular weight of the protein predicted by the open reading frame) and the corresponding genes as *TbMPn* according to the previous convention (Clayton et al. 1998; Panigrahi et al. 2001a). Analysis of protein bands from MAb affinity-purified editosomes identified 16 proteins (Fig. 1D). A representative collision-induced dissociation (CID) spectrum that was obtained from the mass spectrometer from tryptic peptide VLDLEEVYFR is shown in Figure 2A. This peptide corresponds to position 583–592 on a 90 kD protein predicted by *T. brucei* ORF *CHR1.148* (accession no. CAB95444). Thirteen other tryptic peptides were identified that cover 16.8% of the amino acids in this protein (Fig. 2B). We designated this protein TbMP90. Similarly, TbMP100 (DNA clones 20G11, 32N6-TIGR), TbMP99 (ORF *TRYP10.0.000037_106*-Sanger), TbMP67 (ORF *TRYP10.0.000155_38*-Sanger), TbMP61 (ORF

TRYP10.0.000155_52-Sanger), TbMP57 (DNA clones 8B08, 105B11, 9C14-TIGR), TbMP46 (DNA clone 28E9-TIGR), TbMP44 (ORF *TRYP11.0.000049_1*-Sanger), and TbMP24 (ORF *TRYP10.0.000155_8*-Sanger) were identified by identification of multiple tryptic peptides. Sequence analysis of the *TbMP24* and *TbMP99* cDNA clones showed that the second and third AUGs are used as the start codons, respectively. As summarized in Table 1, six editosome proteins that we previously identified by LC-MS/MS, TbREL1, TbREL2, TbMP81, TbMP63, TbMP42, TbMP18 (Panigrahi et al. 2001a, 2001b) and the DEAD box protein mHel61p (Missel et al. 1997), were also identified in purified editosomes by multiple peptide matches. Editosomes purified by the three different methods had similar but not identical protein profiles. Of the 16 proteins detected in MAb affinity-purified complexes, two were not detected in editosomes purified by sequential column chromatography and five were not detected in TAP-tag purified editosomes

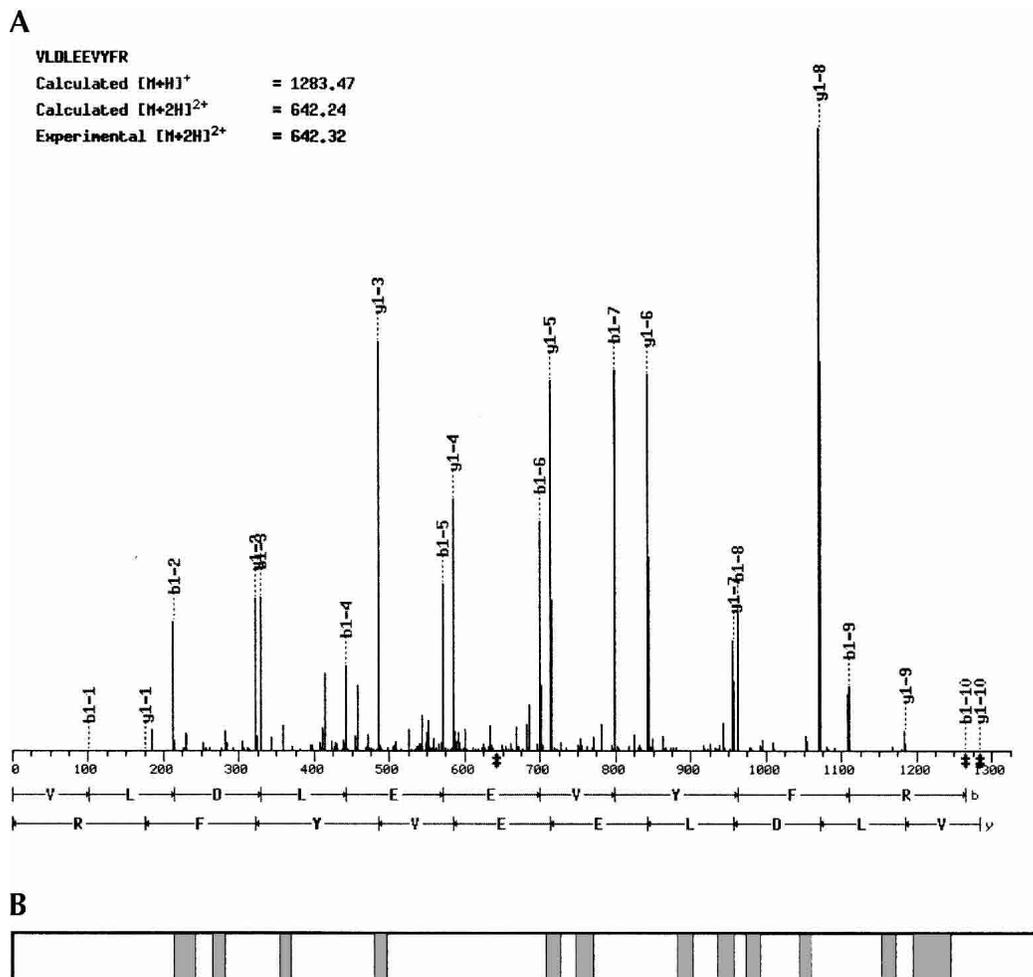


FIGURE 2. Identification of editosome protein TbMP90 by LC-MS/MS analysis. (A) CID spectrum of a tryptic peptide generated by the mass spectrometer. The spectrum matches that predicted for the peptide VLDLEEVYFR, both from N to C terminus (b ions) and C to N terminus (y ions), and corresponds to a peptide predicted from the *CHR1.148* (*TbMP90*) gene sequence. (B) Fourteen tryptic peptides (shaded region, peptide 7 and 8 from left overlap) were identified across the protein that covered 16.8% of the sequence.

TABLE 1. Proteins identified in editosomes isolated by MAb affinity, column chromatography, and TAP-tag procedures

| Protein | MAb affinity | Column | TAP-tag |
|----------------------|--------------|--------|---------|
| TbMP100 ^a | X | X | |
| TbMP99 ^a | X | X | X |
| TbMP90 ^a | X | X | |
| TbMP81 | X | X | X |
| TbMP67 ^a | X | | |
| TbMP63 | X | X | X |
| TbMP61 ^a | X | | X |
| mHel61p | X | X | |
| TbMP57 ^a | X | X | X |
| TbREL1 | X | X | X |
| TbREL2 | X | X | X |
| TbMP46 ^a | X | X | X |
| TbMP44 ^a | X | X | X |
| TbMP42 | X | X | X |
| TbMP24 ^a | X | X | X |
| TbMP18 | X | X | |

^aIdentified in this study

(Table 1). Other editosome proteins that correspond to genes whose sequences are not yet in the *T. brucei* DNA sequence database may be identified from the LC-MS/MS data.

Previous studies showed that the two RNA editing ligase proteins TbREL1 and TbREL2 are related to each other (Panigrahi et al. 2001a; Schnauffer et al. 2001). Four other proteins (TbMP81, TbMP61, TbMP42, and TbMP18) were also shown to have varying degree of sequence similarity and domain conservation among them (Panigrahi et al. 2001b). The editosome protein TbMP24 identified here is related to these four proteins, with the greatest sequence similarity to TbMP42 (29% sequence identity and 42% similarity over 109 amino acids). Similarly, pairwise amino acid sequence comparison and homology searches showed that another set of five proteins, TbMP90, TbMP67, TbMP61, TbMP46, and TbMP44, have some sequence similarity and domain conservation among them (Table 2; Fig. 3A). Of these proteins TbMP90, TbMP67, and TbMP61

are more closely related to each other, as are TbMP46 and TbMP44 (Table 2). Three-way sequence alignment showed the mid region is partially conserved among TbMP90, TbMP67 and TbMP61 (Fig. 3B). Similarly, the mid region of TbMP46 and TbMP44 is partially conserved (Fig. 3C).

Proteins TbMP100 and TbMP99 are similar to each other (overall 28.5/45.8% identity/similarity), and they have a highly conserved C-terminal one-third region (40/61% identity/similarity; Fig. 4). Another novel protein, TbMP57, was identified in editosomes prepared by all three different methods. This protein has sequence similarity to a 108-kD protein 3' TUTase that was cloned from *T. brucei* (Aphasizhev et al. 2002).

The functions of some editosome proteins have been determined whereas the functions of others are suggested by the motifs that they contain (Table 3). TbREL1 and TbREL2 proteins have significant sequence homology to ligase domains and catalyze RNA ligation (McManus et al. 2001; Rusché et al. 2001; Schnauffer et al. 2001). The TbMP81, TbMP63, and TbMP42 proteins contain C2H2 zinc finger motifs (Panigrahi et al. 2001b), implying molecular interaction. The other two proteins in this related set, TbMP24 and TbMP18, contain RNA-binding domains. Thus, this group of five proteins is likely to be involved in protein-protein and/or protein-RNA interactions. Indeed, it has been shown that TbMP63 interacts with TbREL1 (Panigrahi et al. 2001b) and TbMP81 with TbREL2 in vitro (S.S. Palazzo, unpubl.).

The TbMP61 protein has a ribonuclease III motif (amino acids 187–309) located within the sequence that is most conserved with its related proteins TbMP90 and TbMP67. The catalytic center, key residues E and G that function in substrate binding and Mg enhancement of catalysis, respectively, in *Escherichia coli* (Mian 1997) and flanking sequences are conserved (Fig. 3B). Hence, TbMP61, TbMP90, and TbMP67 may function as nucleases. The TbMP44 protein also contains a ribonuclease III-like motif within this region whereas TbMP46 does not, but contains a potential Pumilio family RNA-binding domain motif (amino acids 263–300; Fig. 3C). TbMP44 has been shown to be essential

TABLE 2. Pairwise sequence comparison between editosome proteins

| Protein | Protein | | | | |
|---------------|-----------|-------------|-------------|-------------|-------------|
| | TbMP90 | TbMP67 | TbMP61 | TbMP46 | TbMP44 |
| TbMP90 817 aa | | 32/46 (214) | 27/42 (332) | — | — |
| TbMP67 596 aa | 17.2/28.6 | | 36/52 (287) | 22/46 (118) | 21/44 (198) |
| TbMP61 538 aa | 17.9/26.8 | 23/34 | | 22/37 (275) | — |
| TbMP46 414 aa | 11.3/17.8 | 13.5/23.3 | 17.7/28.6 | | 28/45 (260) |
| TbMP44 382 aa | 10.7/18.8 | 12.8/24.1 | 13.5/22.6 | 21.9/37.8 | |

The global alignment covers the whole length of both sequences and the local alignment covers the region of greatest similarity. The numbers represent percent identity over percent similarity and the length of the region is indicated in parentheses. aa = amino acids.

TABLE 3. Probable and demonstrated function(s) of *T. brucei* mitochondrial editosome proteins

| Protein ¹ | Probable motifs/domains/family ² | Function/probable function ³ | Reference |
|----------------------|--|---|--|
| TbMP81 (a) | C2H2 Zn finger (PS50157) | Macromolecule interaction | Panigrahi et al. 2001b; Drozdz et al. 2002 |
| TbMP63 (a) | C2H2 Zn finger (PS50157) | Macromolecule interaction | Panigrahi et al. 2001b; Huang et al. 2002 |
| TbMP42 (a) | C2H2 Zn finger (PS50157) | Macromolecule interaction | Panigrahi et al. 2001b |
| TbMP24 (a) | S1 domain (PS50126) | RNA interaction | This study |
| TbMP18 (a) | Single-strand binding protein family (PF00436) | RNA interaction | Panigrahi et al. 2001b |
| TbMP90 (b) | Ribonuclease III (PS50142) | Nuclease | This study |
| TbMP67 (b) | Ribonuclease III (PS50142) | Nuclease | This study |
| TbMP61 (b) | Ribonuclease III (PS50142) | Nuclease | This study |
| TbMP46 (b) | Pumilio-family RNA binding (PS50303) | Macromolecule interaction | This study |
| TbMP44 (b) | Ribonuclease III (PS50142) | Structure/Nuclease | This study; B. Wang, in prep. |
| TbREL1 (c) | Ligase (CDD-7281) | RNA ligase | Panigrahi et al. 2001a; Schnauffer et al. 2001 |
| TbREL2 (c) | Ligase (CDD-7281) | RNA ligase | Panigrahi et al. 2001a; Schnauffer et al. 2001 |
| TbMP100 (d) | Endonuclease/exonuclease/phosphatase (PF03372) | Nuclease | This study |
| TbMP99 (d) | Endonuclease/exonuclease/phosphatase (PF03372) | Nuclease | This study |
| TbMP57 | PAP_core, PAP_associated, and Nucleotidyltransferase domains (PS50154, PS50155, PF01909) | Terminal uridylyl transferase | This study; N. Ernst unpubl. |
| mHel61p | DEAD box (PF00270), Helicase_C (PF00271) | Helicase | Missel et al. 1997 |

¹Proteins that show sequence similarity between them have been grouped as a, b, c, and d.

²PROSITE (PS), Pfam (PF), and Conserved Domain Database (CDD) accession numbers for the motifs/domains/family are given in parentheses.

³The proteins with macromolecule interaction function may be involved in RNA-protein and/or protein-protein interactions.

contributed to the editing activity observed by Rusché et al. (1997).

Some proteins with demonstrated or possible roles in editing were not found associated with the stable editosomes described here. These include the 108-kD TUTase (Aphasizhev et al. 2002), gBP21 (Koller et al. 1997; Allen et al. 1998) and the related protein gBP25 (Blom et al. 2001), RBP16 (Hayman and Read 1999), TbRGG1 (Vanhamme et al. 1998), and REAP1 (Madison-Antenucci et al. 1998). This may reflect a low affinity and/or transient association with the editosome or roles associated with processes other than the catalytic steps of editing. Editing probably entails a dynamic series of events that affect protein association with the editosome and perhaps editosome composition (see Stuart and Panigrahi 2002). Hence, some proteins may function in editing but may not be stable components of the editosome or perhaps not even associated with the editosome. Editosomes and editing activities sediment in glycerol gradients with peaks at ~20S and ~40S (Pollard et al. 1992; Piller et al. 1995; Corell et al. 1996), and the relationship between these complexes is unclear. One possibility is that they represent editosomes in various stages of editing and/or association with proteins with roles in editing.

The editing endo- and exonucleases are not yet known although several candidates have been identified in this article. There may be editing endonucleases specific for insertion versus deletion substrates, similar to the TbREL1 and TbREL2 ligases that function in deletion versus insertion

editing (Cruz-Reyes et al. 2002), as well as multiple nucleases to accommodate differences in substrate sequences, gRNA interactions, and positions in the editosome that are produced during the editing of multiple sites. The presence of pairs and sets of related proteins, some with clearly related activities, suggests that insertion and deletion editing are physically and functionally separate. In addition, some essential proteins for editing that are not part of the editosome may or may not directly interact with the catalytic complex. Because editing is regulated during the life cycle of *T. brucei* (Schnauffer et al. 2002), other complexities are bound to be uncovered. Hence, although the steps in editing and the proteins that catalyze these steps are becoming clarified, additional complexities await elucidation.

MATERIALS AND METHODS

Protein and gene identification

Mitochondrial vesicles were isolated from *T. brucei* procyclic cells (strain IsTaR 1.7a) as described (Harris et al. 1990). The mitochondria were lysed with 1% Triton X-100 for 15 min at 4°C with bidirectional mixing, and clarified by centrifugation at 15,000 rpm for 30 min in a microcentrifuge. The soluble sample was fractionated on 10%–30% glycerol gradient, 500 μ L fractions were collected from the top, and the peak fractions positive for editosome as determined by Western analysis were pooled (Panigrahi et al. 2001a, 2001b). Editosomes were then immunoprecipitated from

the pooled sample using anti-TbMP63 MAb. MAbs were incubated with anti-mouse IgG coated Immunomagnetic beads (Dyna beads M-450; DYNAL) for 1 h, washed with IP buffer (10 mM Tris at pH 7.2, 10 mM MgCl₂, 200 mM KCl, 0.1% Triton X-100), and incubated with the mitochondrial fraction. Following 1 h incubation at 4 °C the beads were washed four times with IP buffer. The bound samples to the beads were digested in SDS-PAGE loading buffer and separated on a 10% SDS-PAGE gel. The protein bands were visualized by silver stain. Individual bands were excised from the gel and digested with trypsin. The peptide sequences were analyzed by LC-MS/MS and *T. brucei* nucleotide and protein database searches (Panigrahi et al. 2001a and references cited therein). In cases where complete open reading frames (ORFs) were not available but significant peptide matches were found to genomic sequences, the proteins were identified by further reiterative database analysis and PCR amplification and sequencing of the cDNA and/or genomic DNA clones as described (Panigrahi et al. 2001b). In parallel approaches (a) the editosomes were isolated by sequential SP Sepharose, Q Sepharose, and Superose 6 column chromatography (Panigrahi et al. 2001a) and the proteins were identified by LC-MS/MS analysis of individual SDS-PAGE separated protein bands, and tryptic digestion of the whole complex in solution (Panigrahi et al. 2001b) and (b) the complexes were also isolated by TAP-tag affinity approach as described below and the proteins were analyzed as above.

Cloning and sequencing of the genes

The *TbMP100* ORF was amplified from *T. brucei* genomic DNA (strain 427) by PCR with primers 4430 (ATAAAGCTTATGGCAT TGGCTCAGTCAT) and 4331 (AATGTCGACTTACGGTAA CTTCAATGAAA; restriction sites are italicized), and cloned into pGEM-T Easy vector (Promega) and sequenced. Similarly, the *TbMP99* ORF was amplified by PCR with primers 3275 (ATAG GATCCATGTTGCGCCGAGTCGC) and 3309 (GCGGATCCG AGCTCTAAACCACCTGAAACTC), cloned, and sequenced. *TbMP90* ORF was amplified by primers 4245 (GGAATTCATG CATCATCATCATCATCATTTCCAACCTGGTGCCACATGCG) and 4246 (TAAGCTTTCACGCACCAACCGAGATG); *TbMP67* by primers 4243 (AGGTACCATGCACCATCATCATCATACGC GAAACCTGTCAG) and 4244 (CAAGCTTAGCAAACCTCCAAT GACG), *TbMP61* by primers 4241 (AGGTACCATGCACCAT CACCATCATCATTTTCAGGAGGTGGAGTAC) and 4242 (GAAG CTTAGGGAATGTAATCACTAAAC). *TbMP44* ORF was amplified by PCR with primers 3324 (CGGGATCCCGATGAGAC GGGCTGTGGTAC) and 3325 (CCCAAGCTTGGGTTACCGC CCTCCCAGTGCCAG). The amplified products were cloned into pGEM-T vector and sequenced. RT-nested PCR using primer sets TSL1 (ACTAACGCTATTATTAGAACAG) and 4176 (CGAAA GAACAAACGATATG), followed by TSL2 (GAACAGTTTCTG TACTATATTG) and 4177 (TACTACTAAGTTCAAGAGCTTC) amplified the N terminus of *TbMP46*. The complete ORF sequence was obtained by assembling the sequence of the amplified product with 28E9.TJ sequence.

TAP-tag purification of the editosome

To create a vector for the inducible expression of C-terminally TAP-tagged proteins in *T. brucei*, the tag was amplified from plas-

mid pBS1539 and inserted into plasmid pLew79, generating pLew79TAP. The *TbREL1* coding sequence was released from pLew79-*TbREL1* (Schnauffer et al. 2001) and inserted into pLew79TAP, yielding pLew79-*TbREL1*TAP. *T. brucei* cell line 29.13, coexpressing the TET repressor and T7 RNA polymerase was transfected with *NotI*-linearized pLew79-*TbREL1*TAP plasmid DNA. Phleomycin-resistant clones were selected and checked for tightly tetracycline-regulated expression. Expression of *TbREL1*TAP in the recombinant cell lines was induced for 48 h with tetracycline (10 ng/mL). TAP-tagged editing complexes were purified as described (Rigaut et al. 1999) from 2 L of cells harvested at a density of $\sim 20 \times 10^6$ cells/mL.

In vitro assays

Deletion editing was assayed in vitro using 3' labeled A6-U5 pre-mRNA substrate with gA6[14] Δ 16G gRNA as described (Seiwert et al. 1996). The edited products were detected by polyacrylamide-urea gel electrophoresis and phosphorimaging. *TbREL1* and *TbREL2* were detected by auto-adenylation in the presence of [α -³²P]-ATP as described (Sabatini and Hajduk 1995). The proteins were resolved on 10% SDS-PAGE gels and the radiolabeled proteins were detected by PhosphorImager.

Western blot analysis

The isolated editosome fractions were digested with SDS-PAGE loading buffer and resolved on 10% SDS-PAGE gels. The proteins were transferred onto PVDF membranes, and reacted with MAbs specific for *TbMP81*, *TbMP63*, *TbREL1*, and *TbMP42* (Panigrahi et al. 2001a, 2001b). The filter was developed with ECL kit (Amersham) as per the manufacturer's instructions.

Sequence analysis

The predicted protein sequences were compared with the NCBI nonredundant protein database and EBI database using the BLAST algorithm. The presence of known motifs and domains in the predicted proteins were searched for in the PROSITE, InterPro, BLOCKS, and CDD databases. Homology between the proteins was determined by pairwise sequence comparison using EMBOSS and multiple sequences were aligned using the ClustalW algorithm (www.ebi.ac.uk).

Nucleotide sequence accession numbers

The nucleotide sequences have been submitted to GenBank with accession numbers AY228165–AY228173.

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