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RNA editing produces mature mitochondrial mRNAs in trypanosomatids by the insertion and deletion of uridylicates. It is catalyzed by a multiprotein complex, the editosome. We identified TbMP44 among the components of enriched editosomes by a combination of mass spectrometry and DNA sequence database analysis. Inactivation of an ectopic TbMP44 allele in cells in which the endogenous alleles were disrupted abolished RNA editing, inhibited cell growth, and was eventually lethal to bloodstream form trypanosomes. Loss of TbMP44 mRNA was followed initially by a reduction in the editosome sedimentation coefficient and then by the absence of other editosome proteins despite the presence of the mRNA. Reactivation of TbMP44 gene expression resulted in the resumption of cell growth and the reappearance of editosomes. These data indicate that TbMP44 is a component of the editosome that is essential for editing and critical for the structural integrity of the editosome.
the ability of TbREL1 to compensate for its loss in a non-reciprocal fashion, since these two proteins have considerable sequence similarity. Five other editosome proteins, TbMP18, TbMP24, TbMP42, TbMP63, and TbMP81, of which the largest three have zinc finger motifs, have a complex pattern of sequence relationships (27; R. Salavati et al., unpublished data). TbMP18, TbMP42, TbMP63, and TbMP81 correspond to bands VII, VI, III, and II as designated by the Solnner-Webb laboratory (29). Studies to date (7, 13), as well as those still in progress, indicate that these proteins are essential for editing and show specific interactions with the other components of the editosome as detailed in the Discussion. Importantly, inactivation of TbMP81 gene expression (7) results in preferential loss of TbREL2 and insertion editing while inactivation of TbMP63 (band III) results in loss of TbREL1 (13). This, along with different enzymatic characteristics of the ligase activities, suggests that insertion and deletion editing are functionally and physically separate.

Of the other 14 potential editosome proteins that we identified in purified editosomes, 9 are clearly components of the complex based on a combination of studies that identified or inferred their functions, effect on editing, and association with or relationship to other editosome proteins (25a). The other five proteins are less well studied. One protein, mHel61, encodes an RNA helicase, and its inactivation diminishes editing (24). The incomplete loss of editing shows that mHel61 is not essential for editing and that another helicase, yet to be identified, may also function in editing. A 57-kDa editosome protein has TUTase activity but its requirement in editing is not yet known (34; N. Ernst et al., submitted for publication), while a 108-kDa TUTase is essential for editing (2) but has not been detected in the editosome (25a). Two related editosome proteins, TbMP99 and TbMP100, have endo/exonuclease motifs, while three other related editosome proteins contain RNase III or RNase III-like motifs and are related to two additional related proteins (25a, 34) including TbMP44, which is the focus of this study.

We report here that TbMP44 is an essential component of the editosome since inactivation of TbMP44 gene expression in vivo results in the loss of editing. The inactivation is lethal in bloodstream forms, further indicating that editing is normally essential in this stage of the life cycle. Inactivation is initially followed by structural changes and then loss of editosomes, indicating that TbMP44 plays a role in editosome structural integrity. Reactivation of the TbMP44 gene results in the reappearance of editosomes and the return of editing.

MATERIALS AND METHODS

Cell growth and preparation and analysis of nucleic acids. T. brucei bloodstream forms (strain 427) were grown in HMI-9 medium at 37°C and transferred every 48 h to maintain the log phase growth (12). Genomic DNA was extracted from bloodstream forms by a method adapted from that of Bellotello and Cross (3). Total cellular RNA was prepared using Trizol as specified by the manufacturer (GIBCO-BRL), and contaminating DNA was removed by treatment with RNase-free DNase (Promega) at 37°C. Contaminating DNA was removed by treatment with RNase-free DNase (Promega) at 37°C. The riboprobe was inactivated by heating to 70°C in a water bath followed by structural changes and then loss of editosomes, indicating that TbMP44 plays a role in editosome structural integrity. Reactivation of the TbMP44 gene results in the reappearance of editosomes and the return of editing.

Materials and methods

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Glycerol gradient fractionation. A total of 6 × 10^8 log-phase bloodstream form T. brucei were lysed in 500 µl of IP buffer (10 mM Tris [pH 7.6], 10 mM MgCl2, 300 mM KCl, 1% Triton X-100) containing protease inhibitors (10 µg of leupeptin per ml, 5 µg of pepstatin per ml, 1 mM Pefabloc). The lysates were cleared by a 15-min centrifugation at 13,000 rpm in an Eppendorf 5415D centrifuge at 4°C and then loaded onto 10 to 30% (vol/vol) glycerol gradients. The gradients were centrifuged at 38,000 rpm at 4°C in a Beckman SW40 rotor for 5 h as indicated. Then 500-µl fractions were collected sequentially from the top, flash-frozen in liquid nitrogen, and stored at −80°C for further analysis.

Immunoprecipitation of editosomes. Editosomes were immunoprecipitated as previously described (27). (Mabs) specific for editosome proteins were conjugated to anti-mouse immunoglobulin G-coated immunomagnetic beads (M-450/H9262) and suspended in 50 mM ddGTP was used instead of 0.5 mM dGTP. A 20-mer extension analysis was performed as previously described (8), except that 0.25 PCR products were resolved on 10% polyacrylamide gels. Poisoned primer RPS12, and 3706 (5'-H11032 AACAAAC-3') RNA from each time point was mixed with 5' GTAATGAGTACGTTGTAAAACTG-3' 7 M urea and visualized with a PhosphorImager.

A region (5') of the edited products were separated on 9% polyacrylamide gels precleared deletion editing substrate was U5-5' GPCA6-2A RNA tripartite substrate as previously described (14). The tripartite substrate was 5'-GTAATGAGTAGTACGTTGTAAAACTG-3' for NADH dehydrogenase subunit 4 mRNA (ND4). The reaction was blocked in 5% nonfat milk powder in PBST. After four washes with PBST, the filter was developed using an ECL kit (Amersham) as specified by the manufacturer. Adenylation of RNA editing ligases was assayed as described previously (31). The reaction products were separated by SDS-PAGE (10% polyacrylamide and the radiolabeled proteins were visualized using a PhosphorImager (Molecular Dynamics).

**RESULTS**

Identification of TbMP44. Mass spectrometric analyses of editosomes that were purified by biochemical methods or immunoprecipitated by a MAb specific for editosome proteins were conjugated to anti-mouse immunoglobulin G-coated immunomagnetic beads (M-450/H9262) and suspended in 50 µl of IP buffer to which 50 µl of cleared whole-cell lysate had been added and incubated at 4°C with bidirectional mixing for 1 h. The beads were washed three times with IP buffer and once with HHE (25 mM HEPES [pH 7.9], 10 mM MgCl2, 50 mM KCI, 0.5 mM EDTA) and suspended in 50 µl of HHE.

**Western analysis and adenylation.** Immunoprecipitated editosomes (10 µl) or glycerol gradient fraction (80 µl) was resolved by SDS-PAGE (10% polyacrylamide and transferred onto a nitrocellulose filter. The filter was blocked in 5% nonfat milk powder in PBST (10 mM phosphate buffer [pH 7.2], 150 mM NaCl, 0.1% Tween 20) overnight. The filter was blocked in 5% nonfat milk powder in PBST (10 mM phosphate buffer [pH 7.2], 150 mM NaCl, 0.1% Tween 20) overnight. The filter was then washed three times with PBST and incubated with a cocktail of MAbs against TbMP81, TbMP63, TbRELI, and TbMP32 (diluted 1:50) or anti-hsp70 (diluted 1:1,000) in 5% nonfat milk powder–PBST for 1 h at room temperature. The filter was then washed three times with PBST and incubated with horseradish peroxidase-conjugated anti-mouse immunoglobulin G (Bio-Rad) at 1:2,000 in 5% nonfat milk powder–PBST. After four washes with PBST, the filter was developed using an ECL kit (Amersham) as specified by the manufacturer. Adenylation of RNA editing ligases was assayed as described previously (31). The reaction products were separated by SDS-PAGE (10% polyacrylamide and the radiolabeled proteins were visualized using a PhosphorImager (Molecular Dynamics).

**Assays of RNA editing.** The presence of edited and preedited RNA was assayed by RT-PCR as previously described (33). The upstream and downstream primers for RT-PCR analysis were 3704 (5'-AAAAATAAGTATTTTGATATTATTTAGGATATTAAAG-3') plus 3580 (5'-TATTATATATATATATATGATC-3') for ATPase subunit 6 mRNA, 3705 (5'-ATGACTACATGATAAGTA-3') plus 3601 (5'-CGGAAGACTTAGTCTTACA-3') for NDT, 3619 (5'-CTAATACCTTGGATACAAAC-3') plus 3620 (5'-AAAAACATATCTATATATCAA-3') for RPS12, and 3706 (5'-TGTGTTGACTACAGAGATG-3') plus 3707 (5'-ATCTCTATACCCGCTTA-3') for NADH dehydrogenase subunit 4 mRNA (ND4). The PCR products were resolved on 10% polyacrylamide gels. Poisson primed extension analysis was performed as previously described (8), except that 0.25 mM ddGTP was used instead of 0.5 mM GTP. A 20-mer portion of total-cell RNA from each time point was mixed with 5'-labeled oligonucleotide and polyadenylation sites were mapped (24 and 1392, respectively). Southern analysis shows that TbMP44 is a single-copy gene (right panel). One of the TbMP44 alleles was disrupted in bloodstream forms by targeted gene replacement (39) with a T7 RNA polymerase gene and neomycin (NEO) resistance selectable marker, as diagrammed in Fig. 1A and described in Materials and Methods. Several attempts to replace the second allele were unsuccessful, suggesting that TbMP44 may be essential for survival. Hence, a tetracycline (tet) regulatable ectopic copy of TbMP44 was inserted into the ribosomal DNA spacer region and then the second TbMP44 allele was replaced with the tet repressor (TetR) gene and a hygromycin (HYG) resistance selectable marker (Fig. 1A, left panel). Selection and cloning were done in the presence of tet to enable expression of the ectopic allele. Three clones were obtained with the desired integrations as determined by Southern analysis (right panel), including clone 2C1, which was chosen for further analysis. Wild-type levels of TbMP44 mRNA were present in clone 2C1 in the presence of tet, but the level of this RNA was dramatically reduced within 24 h following the removal of tet from the culture medium (Fig. 1B). Following the removal of tet, clone 2C1 cells continued to grow for about 72 h at a rate comparable to that of cells in the presence of tet (or of wild-type cells). After this time, movement of the cells became sluggish and cell proliferation ceased, until more than 90% of the cells were dead after 144 h (Figure 1C). However, reintroduction of tet 72 h after its withdrawal resulted in a resumption of cell growth at a rate similar to that of the culture from which tet had not been withdrawn. Thus, TbMP44 expression is essential for the growth and survival of bloodstream form T. brucei.

Inactivation of TbMP44 disrupts RNA editing. The effect of inactivation of TbMP44 gene expression on RNA editing in vivo was assessed by RT-PCR and primer extension analysis of total-cell RNA from the clone 2C1 bloodstream forms that was isolated at 24, 48, 72, and 96 h after removal of tet (Fig. 2).
FIG. 1. Inactivation of *TbMP44* gene expression. (A) The left panel shows the strategy for replacement of endogenous *TbMP44* alleles and introduction of a regulatable ectopic allele. Sequences for targeting recombinational replacement (UTR and NTS) are shaded. Constructs were prepared with neomycin (*NEO*), phleomycin (*BLE*), or hygromycin (*HYG*) selectable markers, linearized by *Not*I, and sequentially transfected into bloodstream form *T. brucei* as described in Materials and Methods. Clone 2C1 was confirmed as having the desired genotype. The right panel shows Northern analysis of total RNA and Southern analyses of DNA from wild-type procyclic *T. brucei* 427 (WT) cells and from bloodstream forms of wild-type, transgenic single-knockout (SKO), and transgenic double-knockout with regulatable *TbMP44* allele (DKO + Reg) strains using probes corresponding to the coding region of *TbMP44*, as described in Materials and Methods. RNA and DNA size marker (GIBCO-BRL) positions are indicated. (B) Northern analysis using the same probe as in Fig. 1A, showing the presence or loss of *TbMP44* mRNA in clone 2C1 at various times in the presence of 1 μg of tet per ml (+tet) or after its removal (−tet). Reprobing for α-tubulin mRNA was used as a loading control. (C) Growth of clone 2C1 cells in the presence (solid diamonds) or absence (open diamonds) of tet, or after reintroduction of tet 72 h following its removal (open circles). Cell density was maintained between 0.6 × 10^6 and ~1.5 × 10^6/ml, and the cumulative cell number was normalized according to the dilution factor.
There was a progressive reduction in the abundance of RT-PCR products from fully and partially edited ND7 RNA, A6 RNA, and RPS12 RNA, while PCR products from ND4 mRNA, which does not get edited, remained similar in abundance (Fig. 2A). Pre-edited A6 and RPS12 RNA were more abundant after the removal of tet, probably reflecting their accumulation due to the lack of editing, but this does not appear to be the case for ND7 RNA. Poisoned primer extension of ND7 RNA, which allows for quantitation, revealed ~30 to 40% reduction in edited ND7 RNA after 24 to 48 h, 70% reduction after 72 h, and nearly complete absence after 96 h, all relative to 0 time, while the amount of COI RNA, which does not get edited, remained the same (Fig. 2B). Overall, these results indicate that TbMP44 is essential for RNA editing in bloodstream form T. brucei.

In vitro editing activities that normally sediment at ~20S were essentially absent from clone 2C1 after 72 h in the absence of tet. The endonuclease activity that cleaves a double-stranded editing substrate was essentially absent after 72 h without tet (Fig. 3A). Similarly, precleaved insertion editing, which entails TUTase and ligase reaction pairs, and precleaved deletion activity, which entails exoUase and ligase reaction pairs (14, 15), were essentially absent from clone 2C1 cells after 72 h of growth in the absence of tet (Figs. 3B and C). Some ligated product and a trace of edited product were detected by the precleaved insertion editing assay, but neither of these products was detected by the precleaved deletion editing assay. Substantial exonuclease activity was detected in the precleaved deletion assay and resulted primarily in ~3U product. This is probably due to nucleases other than the editing exonUase in the whole-cell lysates since, as shown in Fig. 3D, fractions 9 to 13 from cells grown with tet primarily removed a single U from the substrate ending in UUAU, reflecting the U specificity of the editing exonUase. However, fractions from cells grown without tet did not stop at the A in the UUAU substrate but primarily removed 3 nucleotides. The absence of editosomes may have left the substrates available for nucleases other than the editing exonUase. The loss of the catalytic activities associated with editing further confirms the role of TbMP44 in editing.

Inactivation of TbMP44 expression disrupts editosomes. Western and adenylation analyses of glycerol gradient fractions from whole-cell lysates revealed that editosomal proteins were essentially absent from clone 2C1 cells after 72 h of growth in the absence of tet (Fig. 4). TbMP81, TbMP63, TbREL1, TbREL2, and TbMP42 proteins cosediment at ~20S from wild-type and clone 2C1 cells grown in the presence of tet (Fig. 4). However, these five editosome proteins were dramatically reduced in abundance following the inactivation of the TbMP44 gene. Signal corresponding to low-molecular-weight proteins was detected at the top of the gradient, suggesting the presence of degraded proteins. Reprobing of the filters with anti-hsp70 antibody revealed that similar amounts of sample were loaded on these glycerol gradients. Thus, the loss of editing is paralleled by the loss of editosomal proteins and hence of editosomes.

Northern analysis of whole-cell RNA revealed that the mRNA for another editosome protein, TbMP63, was retained for at least 72 h following the removal of tet and at levels that were essentially the same as before the removal of tet (Fig. 5). However, the level of TbMP63 protein was substantially diminished within 24 h after the removal of tet and the protein was essentially gone after 72 h. In addition, the level of another editosome protein, TbREL1, was similarly reduced after 24 h and was very low by 72 h. The TbMP44 mRNA level dropped quite rapidly after removal of tet. It was significantly diminished by 2 h, and the mRNA was essentially absent by 4 h (data not shown). TbMP44 protein could not be examined directly due to the lack of a specific antibody. In contrast, while the level of TbMP63 mRNA remained essentially unchanged for 72 h following removal of tet, the level of TbMP63 protein decreased by about 50% within 24 h and then by about 90% within 72 h (data not shown). TbREL1 protein levels decreased with a similar pattern (Fig. 5).

Whole-cell lysates of clone 2C1 that were prepared at various times after withdrawal of tet were fractionated by glycerol gradient sedimentation to assess the consequences of inactivation of TbMP44 expression on editosome integrity. Western analyses using a mixture of four MAbs, which are specific for TbMP81,
TbMP63, TbREL1, and TbMP42, were performed on the gradient fractions, as were adenylation assays, which detect both TbREL1 and TbREL2 (Fig. 6). The editosomes appear intact after 4 h following the withdrawal of tet, since the Western and adenylation results were indistinguishable from those with zero-time and wild-type cells (compare to Fig. 4). However, most of the editosome proteins progressively shifted to the 10S fraction over 24 h. At 36 h, a substantial portion of these proteins were in the 10S fractions but the proportion of proteins in the 20S fractions had increased. After this time, there was a progressive diminution of the protein levels until they were barely detected after 72 h. During this time, hsp70 was readily detectable and its position in the gradient remained similar. Reexpression of TbMP44 by the addition of tet 72 h after its withdrawal resulted in the reappearance of the editosome proteins by 48 h. Examination of cells 72 h after reexpression of TbMP44 revealed proteins distributed primarily between 10S and 20S. These results indicate that TbMP44 is important for editosome integrity.

**DISCUSSION**

This study shows that TbMP44 is a component of the *T. brucei* editosome and is required for editing and for editosome structural integrity. Repression of *TbMP44* expression inhib-
edited the growth of bloodstream forms of *T. brucei* and resulted in loss of editing in vivo, loss of editing-associated catalytic activities in vitro, and perturbation of the editosome structure. *TbMP44* mRNA essentially disappeared between 2 and 4 h after repression of *TbMP44* gene expression (data not shown). The mRNA for another editosome protein, *TbMP63*, remained at apparently normal levels for at least 72 h after *TbMP44* gene repression. However, this protein, other editosome proteins, and editosomes diminished in abundance until they were essentially absent 72 h after *TbMP44* gene repression. This was accompanied by a reduction in the sedimentation coefficient of editosomes. Reexpression of *TbMP44* by reintroduction of tet 72 h after repression by tet removal resulted in a normal growth rate of the culture after a 48-h lag and reappearance of editosomes. These observations indicate that *TbMP44* is essential for functional editosomes and their structural integrity.

The identification of *TbMP44* increases the number of proteins that have been demonstrated to be part of the editosome.

![Western analysis (A) and adenylation assays (B) of glycerol gradient fractions (fraction 1 at the top) from wild-type (WT) cells and clone 2C1 cells grown with tet (+tet) or in its absence (−tet) for 72 h. The Western analyses used a mixture of MAbs specific for the editosome proteins indicated, and the filters were stripped and reprobed with antis-hsp 70 MAb as a loading control. Thyroglobulin (19S) and catalase (11S) were used as sedimentation markers in parallel gradients. See Materials and Methods for experimental details for the Western and adenylation assays.](image)

![Loss of editosomes upon inactivation of *TbMP44* expression. Northern and Western analyses were performed using total-cell RNA or protein. See Materials and Methods for experimental details. Samples were taken from wild-type (WT) cells and from cells grown in the absence of tet (−tet) for the times indicated. Total-cell RNA or protein was taken at 24-h intervals.](image)

To date, over 20 proteins have been identified as candidate components of the editosome (25a). These include the *TbREL1* (*TbMP52*) and *TbREL2* (*TbMP48*) RNA-editing ligases (27), the mHel61p helicase (23), and *TbMP63* and *TbMP81*, which are zinc finger proteins (27) with no known catalytic function but which are essential for the retention of *TbREL1* and *TbREL2*, respectively (7, 13). The 108-kDa 3′ TUTase is essential for editing but has not yet been demonstrated to be in the editosome; however, *TbMP57*, which also has 3′ TUTase activity, is present in the editosome (Ernst et al., submitted). Hence, these proteins may catalyze 3′ U addition to the gRNA and to pre-mRNA, respectively. The editing endonuclease(s) and the 3′ exoUase have not been identified. Other proteins, such as gBP21 (1, 16) and gBP25 (4), RBP16 (11), TbRGG1 (38), and REAP-1 (19), all of which appear to be RNA binding proteins, may play roles in editing, perhaps as accessory factors.

The specific function of *TbMP44* is uncertain, but it is clearly essential for editosome integrity. It contains an RNase III-like motif, which is conserved in its *T. cruzi* and *Leishmania major* orthologs (Salavati et al., unpublished), suggesting that it may be an endonuclease. We could not detect endonuclease or other catalytic activities with in vitro-transcribed-translated protein. Such activities may have been below the sensitivity of the assays due to inadequate protein levels, or its function may require or be enhanced by the presence of other proteins which we have observed for other editing catalysts. Alternatively, *TbMP44* may retain some characteristics of an endonuclease, such as RNA binding. Loss of *TbMP44* does not preferentially affect RNA deletion or insertion editing; rather, all activities are lost, as is editosome structural integrity.

The requirement of *TbMP44* for the structural integrity of the editosome implies a role in editosome stability and/or assembly. About 50% of *TbREL1* and *TbMP63* editosome proteins are lost within 24 h of inactivation of *TbMP44* expression. *TbMP44* mRNA is lost within 2 to 4 h, and *TbMP44* protein levels then must drop to levels that affect function, perhaps within a few hours. Thus, the generation time of ~13.5 h for 2C1 cells suggests that they underwent about one generation between the time when the *TbMP44* protein level became critical and the time when half of the editosome proteins...
were lost. This suggests that there was no net gain or loss of these editosome proteins during this time interval. It implies that either no newly synthesized proteins were incorporated into editosomes or incorporation of newly synthesized proteins was in balance with their rate of turnover. We cannot distinguish between these alternatives at this time, and hence we cannot tell whether TbMP44 plays a role in de novo assembly and/or their stability. However, the appearance of minor bands and changes in relative intensity among the bands in the Western analyses (Fig. 6) imply that some protein degradation is occurring within the 24-h period.

The shift of most editosomes from \( \sim 20S \) to \( \sim 10S \) by 24 h, indeed by 12 h when the levels of TbREL1 and TbMP63 were reduced by only \( \sim 25\% \), suggests the involvement of a more dynamic process than de novo assembly and/or editosome protein turnover. Such a process might entail a dissociation of editosome subunits and/or accessory factors. This shift is intriguing in view of the accumulating evidence that insertion editing and deletion editing are functionally and physically separate. This separation was first implied by the presence of two editing ligases (21, 27, 29, 31) that have somewhat distinct catalytic characteristics (6, 13, 30). It is further supported by the preferential loss of TbREL2 upon TbMP81 gene inactivation (7) and of TbREL1 upon TbMP63 gene inactivation (13). Indeed, recent studies indicate the existence of editosome subunits for insertion and deletion editing (A. Schnaufer et al., submitted for publication). These studies strongly support the separation of these two types of editing. One possibility that we suggest is that the insertion and deletion editosome subunits separate during the editing process, perhaps after each gRNA is used, and that TbMP44 is essential to the reassembly with another gRNA. These possibilities are currently under assessment.

The reproducible relative increase in the level of \( \sim 20S \) editosomes 24 h after TbMP44 gene inactivation, which is more evident at the 36-h time point, implies the occurrence of a second process. One possibility is that another protein may substitute for the assembly, stability, or subunit interaction function of TbMP44. A candidate for this substitution is TbMP46, which has significant sequence identity and similarity

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**FIG. 6.** Editosome changes resulting from inactivation of TbMP44 expression. Glycerol gradient fractions of whole-cell lysates from samples were taken after the removal of tet at the times indicated and 48 h after the addition of tet to cells at 72 h after removal of tet. The gradient fractions were assayed by Western analysis using a mixture of four MAbs specific for the TbMP81, TbMP63, TbREL1, and TbMP42 editosome proteins (A) and by adenylation that detects the TbREL1 and TbREL2 editing ligases (B). The filters were stripped and re-probed with anti-hsp70 MAb as a control. See Materials and Methods for details.
to TbMP44 (25a). Such a substitution may not result in functional editosomes and hence may ultimately lead to editosome loss and cell death. An alternative possibility is that the shift to a larger S value might reflect an association with protein-processing machinery. Overall, the repression of TbMP44 expression results in the eventual diminution and loss of editosomes and editosome proteins. Thus, despite the specific role of TbMP44, it is essential for the production and/or retention of editosomes.

The rescue of cells from death and the reacquisition of editosomes upon reactivation of TbMP44 gene expression after 72 h indicate that the temporary loss of editosomes was not lethal and that the loss of editosomes is due to inactivation of TbMP44 expression. The difference in the sedimentation profile of the editosome proteins in the rescued cells compared to the zero-time and wild-type cells may reflect the presence of dead or dying cells in the 72-h sample and/or the presence of partially assembled editosomes that follow the lag in returning to the normal growth rate following reactivation of TbMP44 gene expression.

The inhibition of growth of bloodstream forms upon inactivation of TbMP44 expression supports the suggestion that editing is normally required in bloodstream forms (33). Inactivation of other editosome protein genes, including the TbRELI editing RNA ligase (33) and the TbMP81 (7) zinc finger protein, also is lethal in bloodstream forms, as is inactivation of mt topoisomerase II, which creates mutants lacking mt DNA (32). This reduces the possibility that editosome proteins are multifunctional and that lethality is due to functions not associated with editing. The finding that editing is normally essential in bloodstream forms suggests that the significance of mt gene expression in this life cycle stage, e.g., for generation of ATP (10) and other potentially vital functions, is incompletely understood. These forms lack cytochromes and were thought to rely solely on glycolysis for ATP production. There is no evidence that glycolytic enzyme production requires RNA editing. However, NADH dehydrogenase complex and/or ATPase complex, both of which reside in the inner mt membrane and contain subunits translated from edited mRNA, may have functions essential for the bloodstream stage. Mutants that do not edit normally, if at all, since they lack all or most kDNA, appear to have compensated for this loss (6a, 32).

Overall, TbMP44 was shown to be a component of the editosome that is critical for editosome function and editosome assembly and/or structural integrity. No catalytic function was identified, but it may play a role in dynamic processes that occur during editing. It is normally vital for bloodstream form T. brucei and has homologs in other pathogenic trypanosomatids and thus may be a potential drug target for chemotherapy for African sleeping sickness and related diseases.

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