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Mitochondrial RNA editing in trypanosomes: small RNAs in control

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Abstract

Mitochondrial mRNA editing in trypanosomes is a posttranscriptional processing pathway whereby uridine residues (Us) are inserted into, or deleted from, messenger RNA precursors. By correcting frameshifts, introducing start and stop codons, and often adding most of the coding sequence, editing restores open reading frames for mitochondrially-encoded mRNAs. There can be hundreds of editing events in a single pre-mRNA, typically spaced by few nucleotides, with U-insertions outnumbering U-deletions by approximately 10-fold. The mitochondrial genome is composed of ~50 maxicircles and thousands of minicircles. Catenated maxi- and mini-circles are packed into a dense structure called the kinetoplast; maxicircles yield rRNA and mRNA precursors while guide RNAs (gRNAs) are produced predominantly from minicircles, although varying numbers of maxicircle-encoded gRNAs have been identified in kinetoplastids species. Guide RNAs specify positions and the numbers of inserted or deleted Us by hybridizing to premRNA and forming series of mismatches. These 50-60 nucleotide (nt) molecules are 3' uridylated by RET1 TUTase and stabilized via association with the gRNA binding complex (GRBC). Editing reactions of mRNA cleavage, U-insertion or deletion, and ligation are catalyzed by the RNA editing core complex (RECC). To function in mitochondrial translation, pre-mRNAs must further undergo post-editing 3' modification by polyadenylation/ uridylation. Recent studies revealed a highly compound nature of mRNA editing and polyadenylation complexes and their interactions with the translational machinery. Here we focus on mechanisms of RNA editing and its functional coupling with pre- and post-editing 3' mRNA modification and gRNA maturation pathways.

Keywords

trypanosoma; mitochondria; RNA editing; RNase III; TUTase; RNA ligase; RNA binding proteins; protein complexes; cryptogenes

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1. Introduction

The discovery of RNA editing in *Trypanosoma brucei* by Rob Benne and co-workers [1] paved the way to understanding a fundamentally novel mechanism of information transfer between RNA molecules and illuminated a much greater coding capacity of the mitochondrial genome than had been predicted from DNA sequencing. Twelve mitochondrial genes that seemed nonfunctional or nonexistent were identified as cryptogenes whose transcripts must be post-transcriptionally decoded (edited) by inserting or deleting Us in order to produce open reading frames. Initially perceived as a challenge to the central dogma of molecular biology, the editing phenomenon stimulated search for a template ultimately leading to the discovery of guide RNAs (gRNAs) [2]. By allowing for wobble G-U, in addition to canonical Watson–Crick base-pairing, short (50-60 nt) mitochondrial transcripts have been recognized as complementary to edited sequences and, therefore, likely carriers of genetic information. Partial annealing of gRNAs and pre-edited mRNAs immediately suggested a mechanism by which the location and extent of U-insertions and U-deletions are determined [2]. The site selection for gRNA binding is accomplished via short region of complementarity between gRNA's 5' "anchor" region and mRNA; the rest of gRNA forms an imperfect duplex along the mRNA by either bulging out uridines in mRNA, or adenines and guanosines in gRNA. Unpaired Us in the pre-mRNA are removed, and unpaired purines in the gRNA specify insertion of an equal number of Us into opposing mRNA positions (Fig. 1). In massively (pan) edited mRNAs editing events proceed sequentially in 3' to 5' polarity along the mRNA and require multiple overlapping gRNAs [3]. The overall fidelity of the editing process is astonishingly low with the bulk of the mRNA population represented by partially-edited or miss-edited transcripts, which raises the problem of how correctly edited mRNAs are selected for ribosome binding and translation.

The narrow phylogenetic distribution of U-insertion/deletion editing, which is limited to kinetoplastids protozoans, is indicative of its origination within a particular lineage rather than being a trait shared with the common ancestor of eukaryotes. There are, however, signs that RNA editing may have arisen consequential to acquisition of novel functions by primordial cellular enzymatic modules involved in DNA/RNA repair and RNA interference. The sheer complexity of the editing machinery and its intertwinement with pre- and post-editing mRNA polyadenylation and translation raise exciting questions about how RNA editing systems appeared and became fixed in evolution [4;5].

2. Basic mechanism of U-insertion/deletion mRNA editing and activities of the core editing complex

The "enzymatic cascade" model [2] was confirmed by reproducing elementary reactions and complete editing cascade in a single site with synthetic mRNA and gRNA as substrates and crude mitochondrial extract as the source of editing complexes [6;7]. Further studies of purified RNA editing core complex (RECC, also referred to as the ~20S editosome) identified specific components responsible for each enzymatic step and revealed substrate specificities of individual enzymes (reviewed in [8], Table 1). Remarkably, the information transfer from gRNA to mRNA does not involve an RNA-dependent recognition of the

incoming UTP as would be the case in a typical template-copying polymerization reaction. Instead, intrinsic substrate specificities of key enzymes, such as UTP selection by RET2 terminal uridylyltransferase (TUTase) [9], are responsible for the overall fidelity of editing.

Editing is initiated by an endonucleolytic pre-mRNA cleavage at the first unpaired nucleoside adjacent to the continuous ‘anchor’ duplex, which is a bulged out uridine at the deletion site or typically a purine base in the insertion site (Fig. 1). Cleavage reaction generates 5' and 3' mRNA cleavage fragments that are presumably tethered by hybridization with gRNA and, most likely, RNA-protein contacts with the RNA editing core complex and/or the gRNA binding complex (GRBC [10], reviewed below). The importance of maintaining mRNA cleavage fragments bound to gRNA has been illustrated in “pre-cleaved” assays that recapitulate a three-RNA hybrid product of an endonucleolytic cleavage (Fig. 1, step II). Specifically, introduction of extended complementarity regions between gRNA and both cleavage fragments stimulated U-insertion, U-deletion and RNA ligation reactions [11-14].

The asymmetrical structures of U-deletion and U-insertion sites are distinguished by RNase III-type endonucleases, REN1 [15] and REN2 [16], respectively. The third endonuclease (REN3) apparently targets the COII mRNA that contains a *cis*-acting guide RNA-like element in its 3' untranslated region (UTR) [17;18]. Remarkably, while most RNase III catalytic domains form homodimers with two active sites and cleave both strands in the double-stranded RNA, only the pre-mRNA strand is cleaved during editing. To account for a single cleavage event, Carnes et al. suggested that editing endonucleases REN1 and REN2 may form heterodimers with RNase III-like, but catalytically inactive RECC components (MP46 and MP44, Table 1) respectively, thus leaving only a single functional active site [17]. Furthermore, a mutually exclusive association of REN1, REN2 and REN3 with common set of proteins that contains U-deletion, U-insertion and ligase activities pointed to the modular nature of editing complexes [17-20]. In a simplest model, an endonuclease module, e.g., an endonuclease associated with a specific protein(s) such as REN1/REX1/MP41, REN2/MP47 or REN3/MP49, would bind to a common particle to confer specificity for U-deletion, U-insertion or *cis*-edited sites, respectively (Fig. 1). Thus, the enzymatic RNA editing core complex exists in at least three isoforms that share most of their subunits, but can be distinguished by the presence of site-specific endonuclease modules.

Within the common set of proteins, seemingly opposing U-deletion and U-insertion cascades are spatially separated such that key enzymes are arranged around distinct structural proteins. Zinc-finger (C2H2)-containing proteins MP63 and MP81 emerged as principal organizers for U-deletion and U-insertion subdomains, respectively [21-24]; MP63 is engaged in direct protein-protein interactions with REX2 U-specific 3'-5' exonuclease and REL2 RNA ligase, whereas MP81 forms extensive contacts with RET2 TUTase and REL1 RNA ligase. Both REX1 and REX2 exonucleases possess exonuclease-endonuclease-phosphatase (EEP) catalytic domains manifested by their indistinguishable exonuclease activities that are specific for single-stranded uridines [20;25]. In *T. brucei*, however, exonuclease' distribution within the core editing complex is remarkably distinct: the essential for editing REX1 is associated with REN1 endonuclease module, and likely represents the main U-deletion activity; the dispensable REX2 seems to play a structural role

in the U-deletion subdomain [25;26]. Accordingly, REX2 is missing a catalytic domain but remains associated with U-deletion subdomain in closely related organism *Leishmania tarentolae* [25;27;28].

In the U-insertion subdomain, RET2 TUTase interacts with MP81 zinc finger proteins via a non-catalytic middle domain resulting in mutual stabilization and stimulation of TUTase activity on double-stranded RNA substrates [9;24;29-31]. RET2's exclusive selectivity for UTP is determined by a highly-structured binding site that accommodates the uracil base via a network of direct and water-mediated hydrogen bonds, and stacking interactions [9]. Because guide RNA plays no role in UTP selection, both adenosines and guanines in guiding positions direct U-insertion editing with equal efficiency. Conversely, the RET2's RNA substrate specificity plays an important role: a single uridine may be added to the mRNA cleavage fragment with similar efficiency whether or not this +1U would base pair with gRNA. However, if a mismatch occurs between the newly added +1U and the gRNA, addition of the next U to a single-stranded overhang is blocked because of enzyme's strong preference for double stranded RNA [29]. Furthermore, RET2 TUTase adds Us in different modes depending on the chemical nature of the last mRNA-gRNA base pair. A distributive +1 addition is very prominent when a purine base occupies the 3' nucleotide of the mRNA 5' cleavage fragment (Fig. 1, step II) while virtually no +1 addition takes place if the RNA substrates terminates with U; instead, RET2 processively fills the gap as defined by the number of guiding nucleotides. Although the rates of +1U addition are similar between recombinant RET2 and purified RNA editing core complex, the *in vitro* gap-filing capacity of the latter substantially higher (up to 10 Us) whereas the former is limited to 4-5 Us. The RET2's substrate is generated by the endonucleolytic mRNA cleavage which leaves a monophosphate group at the 5' end of the 3' mRNA cleavage fragment (Fig. 1). The phosphate, however, is not required for U-insertion by the editing complex, but is essential for recombinant RET2's activity. This may reflect mechanistic similarities between RNA editing and base excision DNA repair enzymes. Gapped DNA intermediates generated during base excision DNA repair by AP endo/exonucleases, which are homologous to the REX1/REX2 editing exonucleases, are topologically similar to expected post-cleavage RNA editing intermediates. Such DNA lesions are repaired by the DNA polymerase β , a founding member of the nucleotidyl transferase superfamily to which RET2 belongs [25;32]. The DNA repair polymerase activity strictly depends on the 5' phosphate recognition [33] to processively, albeit with low fidelity, read through the lesion as long as the gap does not exceed 4-5 nucleotides. The phosphate group requirement for RET2-catalyzed reaction indicates that the double-stranded "anchor" with an internal 5'-monophosphate constitutes the RET2 binding site. In the case of RECC, the lack of phosphate dependence and capacity to fill longer gaps points out the contribution of other subunit(s) to RNA binding. If RET2, as core complex subunit, remains bound to the "anchor" duplex upon mRNA cleavage, the 3'-OH group of 5'-mRNA cleavage fragment must be somehow positioned in the vicinity of the active center. For short gaps, the 3'-hydroxyl group may be held in sufficiently close proximity by 3-4 guiding nucleotides; hence efficient U-insertion may not require additional RNA contacts outside of RET2. For longer gaps, a greater entropy cost of bringing the 3'-OH group into the RET2's active site is likely to be borne by interactions with other RECC subunits.

Both U-deletions and U-insertions produce a double-stranded RNA in which mRNA fragments are separated by a nick and complementary gRNA acts as a bridge, an optimal substrate for RNA editing ligases [14]. RNA editing ligases 1 and 2 (REL1 and REL2) have been identified as components of U-deletion and U-insertion subdomains, respectively [22;24;28]. Although spatial separation and contacts with distinct structural proteins within these subdomains, MP63 and MP81, respectively [21;23;27;34] provide a strong case for specialized roles, only REL1 was found to be essential for cell viability [35-39]. Remarkable similarities of catalytic domains structures and RNA substrate specificities between trypanosomal RNA editing ligases and T4 RNA ligase 2 (Rnl2) [40-42] further contribute to the notion of primordial RNA/DNA repair systems as potential evolutionary source of editing activities.

3. Auxiliary RNA editing complexes

The three isoforms of RNA editing core complex are responsible for elementary editing reactions and mostly likely target the majority of editing sites in mitochondria of trypanosomes. Over the last five years it has become obvious that the complexity of the editing pathway and its entangled relationships with pre- and post-editing processing events and translation extend far beyond basic enzymology of mRNA cleavage, U-insertion and deletion, and RNA ligation. Below we review more recent findings that uncovered key players in processing and stabilization of RNA editing substrates (pre-edited mRNAs and guide RNAs), and mRNA 3' modification.

3.1. Guide RNA processing

The distinct features of gRNA termini, 5' triphosphate and 3' oligo(U) tail, were recognized soon upon gRNA discovery [2;43] and these findings suggested the absence of 5' processing and the existence of 3' degradation and uridylation pathway. The RNA terminal uridyltransferase activity was detected in mitochondria of *Leishmania tarentolae* [44] and the responsible protein was purified by conventional methods [45]. Termed RNA Eding TUTase 1 (RET1), this enzyme was initially associated with gRNA uridylation based on RNAi knockdown studies that demonstrated gRNA's shortening and concomitant decrease in abundance, and an inhibitory effect on editing *in vivo* [24;45]. The U-tail's participation in the editing process (direct effect) and/or its requirement for gRNA stabilization in mitochondria (indirect effect) were initially considered as potential causes of efficient editing blockade upon RET1 repression. Indeed, interactions of the U-tail with purine-rich pre-edited mRNA may serve a mechanistic purpose of stabilizing gRNA-mRNA hybridization [43], and such effect was observed for synthetic RNAs in the absence of proteins [46-48]. In addition, the U-tail may function as a binding platform for editing complexes. To that end, several proteins with pronounced affinity for poly(U) have been identified, but genetic repression of neither had a uniform inhibitory effect on the editing process [49-52] nor was the U-tail required for *in vitro* editing reactions [53]. Although participation of the U-tail in the editing process remains entirely possible, a more detailed study presented this structure as disposable for gRNA stability. Conversely, the loss of mature gRNAs in RET1-depleted parasites was attributed to impaired gRNA precursor processing [54]. In *T. brucei* gRNAs are transcribed predominantly from minicircles as

uniformly-sized (~800 nt) precursors [55]. Because ~1 kb-minicircles typically possess 3-5 gRNA genes these precursors are likely to be polycistronic and since 5' triphosphates are found on mature ~60 nt gRNAs it would be plausible to assume that only the most 5' gRNA is processed into mature uridylated molecule while the long 3' trailer is removed prior to uridylation. These observations raise more questions of which the most pressing are: 1) Why the knockdown of RET1 protein or repression of its TUTase activity by a dominant negative mutation inhibited the nucleolytic degradation and 2) What is the mechanism of a relatively precise endonucleolytic cleavage, or 3'-5' exonucleolytic degradation blockade, that generate gRNA's 3' end prior to uridylation. On a first account, it seems possible that RET1 TUTase may form a complex with a nuclease of interest and that the nuclease activity is inhibited in the absence of RET1. Uridylation of long gRNA precursors as a prerequisite for efficient exonuclease recruitment would be also consistent with available data and reminiscent of uridylated pre-*let-7* miRNA precursor degradation by the Dis3l2 exonuclease in mammalian cells [56]. Ultimately, a detailed characterization of macromolecular complexes involving RET1 [24;45] and sequencing of gRNA precursors would be essential to further progress in this direction. In regards to the cleavage mechanism, a model has been proposed in which anti-sense uridylated transcripts originating from the opposite strands in the vicinity of gRNA gene direct the pre-gRNA degradation [54;57]. More specifically, a double-stranded region presumably formed by 5' regions of long antisense transcripts may either guide an RNase III-like endonuclease activity, not dissimilar to editing endonucleases (Fig. 1), or block 3'-5' exonuclease degradation. Although a candidate RNase III-type mitochondrial RNA processing endonuclease 1 (mRPN1) endonuclease has been proposed to fulfill the processing function, the length of gRNA precursors observed by Madina et al as accumulating in mRPN1 knockdown [58] was inconsistent with those detected in RET1 knockdown [54]. Although further work is required to understand the mechanism of gRNA processing, the existence of a double-stranded intermediate, if confirmed, would further corroborate evolutionary links between U-insertion/deletion RNA editing and RNA interference.

With editing in mind, one must also consider the presence of gRNA-like small RNAs detected in mitochondria of *L. tarentolae* [59] and *T. brucei* [60]. The distinction with gRNAs is quite formal at this point with fully-edited mRNAs serving as the main criteria: if a small mitochondrial RNA can be annealed to a single edited mRNA, with G-U base pairing allowed, and account for known editing patterns, then it is considered to be a gRNA. Because most gRNA-edited mRNA hybrids still contain mismatches this approach leaves much leeway with setting cutoffs for the number of allowed imperfections. Such predictions are further hampered by the lack of experimental knowledge about thermodynamics of binding necessary for initial anchor-target recognition and sufficient to support the cascade of editing reactions. Although most gRNAs are predicted to specify editing of multiple adjacent sites it is unclear whether the entire informational capacity of each gRNA is realized. In particular, editing events specified by the 3' portion of gRNA would require the 5' mRNA cleavage fragment to be tethered by a very limited number of base pairs (Fig. 1). Theoretically, this potential problem may be overcome by either stabilizing involvement of protein complexes or by participation of overlapping gRNAs that may outcompete or displace the 3' region of already bound gRNA. This scenario would also imply that certain

criteria are imposed by editing endonuclease on the length and/or stability of double-stranded regions surrounding the editing site. Finally, as pointed out by Hajduk and colleagues, binding of gRNA-like molecules to pre-edited mRNAs may generate alternative editing patterns and potentially lead to synthesis of a different polypeptide [61;62]. Although considering the U-insertion/deletion editing as a source of protein diversity remains a wide-open area, there is a high likelihood that gRNA-like molecules are synthesized and processed in the same pathway as canonical gRNAs. Their functions in other elements of mitochondrial gene expression, such as nucleolytic processing of multi-cistronic maxicircle transcripts and regulation of translation, await further studies.

3.2. Guide RNA binding and stabilization

The concept of small RNAs transitioning between protein complexes from transcription to function to decay is virtually universal and guide RNAs are no exception. The early work on gRNA-containing complexes indicated that 60-65 nt-long gRNAs participate in ribonucleoprotein particles ranging from 10S to 50S [63-65]. Several candidate gRNA binding proteins have been identified by various biochemical means and investigated for their roles in RNA editing, but these studies were confounded by the lack of conclusive genetic data and uniform effects on editing [50;51;66-74]. In the most prominent case of MRP1/2 complex, many findings (high affinity for gRNA, capacity to promote the annealing of complementary RNAs, downregulation of editing for the cytochrome *b* mRNA upon dual MRP1/2 repression) pointed to some function in mitochondrial RNA processing, but did not identify a specific role in editing. However, two proteins isolated from *L. tarentolae* by co-purification with MRP1/2 [71] fit the profile for an essential component of gRNA biogenesis [71]. Termed gRNA binding complex subunits 1 and 2 (GRBC1 and GRBC2, also referred to as GAP2 and GAP1, [75]), these polypeptides assemble into stable $\alpha_2\beta_2$ heterotetramer and bind gRNA *in vivo* and *in vitro*, and their knockdowns led to a global gRNA destabilization and inhibition of editing [10;54]. The knockdowns of either individual subunit led to the reciprocal loss of a protein binding partner [75]. The GRBC1/2 tetramer is engaged into stable protein-protein interactions with several proteins lacking discernible motifs, which together constitute a part of a still larger ribonucleoprotein assembly to which we will refer as the gRNA binding complex (GRBC). This complex has also been named mitochondrial RNA binding complex 1 (MRB1, [76], reviewed in [77]). In addition, GRBC1 and 2 were detected in the affinity-purified polyadenylation complex [78] and large ribosomal subunit [79]. Although studies of the guide RNA binding complex are still in their early stages, some conclusions can be drawn at this point: 1) The number of GRBC subunits is likely to exceed that of the RNA editing core complex; 2) The GRBC1/2 tetramer appears to be the guide RNA binding interface of a GRBC, which is also involved in mRNA binding, interactions with RECC, polyadenylation complex and the ribosome [79]; 3) The GRBC complex is likely to have a modular composition with GRBC1/2 being part of one stable module and RGG2 RNA binding protein representing a subunit of another module [80;81]; 4) Most GRBC subunits are essential for the RNA editing process, but are not required for gRNA stabilization and 5) Both protein-protein and RNA-mediated interactions are crucial for GRBC integrity and function. The nearly identical outcomes of RNAi knockdown experiments for GRBC-associated proteins (inhibition of editing and cell growth, but no effect on gRNAs) and affinity purifications of the same (identification of still

more candidate proteins) underscore the enormity of challenge to understand the inner works of this complex [80;82;83]. The question of gRNA recognition by GRBC1/2 becomes particularly important in light of hazy distinction between gRNAs and gRNA-like molecules. If gRNA processing indeed involves a double-stranded intermediate, the question of selective gRNA loading onto GRBC (effector complex) and metabolic fate for the antisense (passenger) RNA strand immediately arise. With application of *in vivo* cross-linking based methods and deep sequencing approaches we are bound to witness new insights in a near future.

3.3. Remodeling of mRNA-containing macromolecular complexes

At this stage in our understanding of the editing process, we assume that: 1) The double-stranded RNA is formed during initiation and extended by progression of editing; 2) The post-editing unwinding of gRNA-mRNA duplexes must take place to liberate a binding site for the next gRNA; 3) Fully-edited mRNAs and gRNAs are predicted to form stable hybrids that are likely to impede translation unless displaced by an active mechanism; and 4) mRNA-containing ribonucleoprotein complexes undergo remodeling during transcription, editing and translation. Participation of RNA helicases in the U-insertion/deletion editing process is as definitive as elusive are the specific roles of these NTP-hydrolysis driven molecular remodelers. To date, genetic repression of two (DExH)-box proteins has been shown to reduce production of edited mRNAs albeit in a transcript specific fashion: mHel61p [84], renamed RNA editing helicase 1 (REH1) [85], and RNA editing helicase 2 (REH2) [75;86]. REH1 has been detected in some preparations of the core editing complex [87] while REH2 co-purifies with GRBC (MRB1) complex via RNA link [10;74;75;86]. For both helicases, the RNA-mediated interactions with respective editing complexes are stable enough to withstand affinity purification. The mechanistic details of REH1's involvement in editing are beginning to emerge with evidence of its participation in the ATP-dependent displacement of sequential gRNAs *in vitro* [88]. Two independent studies applied labeling of primary transcripts with vaccinia virus guanylyl transferase to detect a REH2 RNAi-triggered decrease of guide RNA abundance [75;86]. These results, however, remain to be verified by alternative methods because a 5' triphosphorylated mitochondrial RNAs include not only gRNAs, but also gRNA-like molecules [59;60]. In addition, the guanylyl transferase-based detection reflects a collective 5' phosphorylation state of rather than RNA abundance. It is, however, entirely possible that REH2 may function in disengaging fully-edited mRNAs from editing complexes prior to further processing.

4. Integrating RNA editing with pre- and post-editing processing events

Following excision from multicistronic precursors, mitochondrial pre-mRNAs typically possess short 5' monophosphorylated UTRs without apparent ribosome binding sites and short 3' UTRs. Mitochondrial mRNAs are also 3' modified in a rather convoluted manner: pre-edited mRNAs typically possess 20-25 nt A-tails while fully-edited mRNAs can be separated into populations bearing short A-tails and those with short A-tails extended into 200-300 nt-long A/U-tails. Unedited mRNAs are also split into fractions with short A-tails and long A/U-tails [78;89-92]. Importantly, pre-edited mRNAs are 3' modified by the short A-tail addition prior to initiation of editing and remain such for the duration of editing

process as it proceeds in the 3'-5' direction. The long A/U-tails composed of stretches of As interspersed by few Us are added to the pre-existing 3' A-tails upon completion of the editing process, which typically occurs at the mRNA's 5' region. Identification of mitochondrial poly (A) polymerase KPAP1 [78] and polyadenylation/ uridylation factors KPAF1 and KPAF2 [79] enabled functional analysis of 3' modifications and revealed intertwined relationships between mRNA polyadenylation, editing and translation. KPAP1 repression led to a decline of both short- and long-tailed forms of unedited and fully-edited mRNAs followed by rapid cell death. To the contrary, the steady-state levels of pre-edited mRNAs were not affected by KPAP1 knockdown and ensuing loss of short A-tails [78]. These findings clearly implicated the pre-editing addition of the short A-tail in stabilization of partially- and fully-edited mRNAs, but raised more questions: 1) What is the mechanism of the short A-tail's function "switching" from neutral in pre-edited to stabilizing in edited mRNAs; 2) What is the nature of signaling between completion of U-insertion/ deletion editing at the 5' region and A/U-tail addition to the 3' end; and 3) How is the A/U tail built and what is the function of this unusual structure.

Transient association of RET1 TUTase with KPAP1 [78] and RET1's requirement for UTP-stimulated mRNA degradation *in organello* [93;94] implicated this enzyme as possible KPAP1 counterpart in the mRNA adenylation/uridylation process. However, attempts to reconstitute A/U tail synthesis *in vitro* with recombinant KPAP1 and RET1 failed [78] until a purified complex of two pentatricopeptide repeat-containing (PPR) proteins, initially discovered in the polyadenylation complex and termed **k**inetoplast **p**oly**a**denylation/ uridylation **f**actors (KPAFs) 1 and 2, was added to the reaction [79]. RNA binding PPR proteins are defined by the presence of degenerate 35-amino acid tandem repeats [95] and are highly abundant in terrestrial plants with hundreds of non-redundant factors controlling organellar RNA processing and translation [96]. In *T. brucei*, more than 40 PPRs, including those associated with the KPAP1 complex [78;79] and mitochondrial ribosomes [97] have been annotated (reviewed in [98]). At least some ribosome-associated PPRs are essential for rRNA biogenesis or stability, as evidenced by the loss of either 9S or 12S rRNAs in respective RNAi knockdowns [99]. In agreement with their proposed function, knockdown of KPAF1 and 2 led to selective elimination of the A/U-tailed mRNAs while leaving short A-tailed mRNAs unaffected [79]. Furthermore, edited transcripts bearing 200-300 nucleotide-long A/U-tails, but not short A-tails, were found preferentially associated with small ribosomal subunits in translating ribosomal complexes [79;100;101]. To conclude, initial studies established the KPAP1-catalyzed pre-editing short A-tail addition as required and sufficient for mRNA stabilization during and after the editing process. Conversely, the KPAF1-2 coordinated post-editing A/U-addition by RET1 and KPAP1 represents a final step in biogenesis of mitochondrial translation-competent mRNAs in *T. brucei*. It appears that short A-tailed and A/U-tailed mRNA are equally stable, but only the latter form binds to the ribosome and participates in translation [79].

5. Outlook

The complexity of ribonucleoprotein complexes performing mRNA editing reactions, delivering editing substrates, stabilizing intermediates and channeling products is further exacerbated by their dynamic interactions and coupling with pre- and post-editing

processing events, and translation. Although structure-function studies of RECC and GRBC are progressing at a measured pace and more participants are being uncovered, there is a dearth of knowledge of how specificity of editing is achieved in the light of exceedingly complex small RNA transcriptome in mitochondria of trypanosomes. These questions beg for experimental analysis of minicircle genome and exhaustive mapping of small RNA genes, their promoters and termination signals. It is conceivable that a pervasive but precisely initiated transcription would produce both gRNA precursors and antisense molecules acting as chaperones of their processing.

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References

1. Benne R, Van den Burg J, Brakenhoff J, Sloof P, Van Boom J, Tromp M. Major transcript of the frameshifted *coxII* gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. *Cell*. 1986; 46:819–826. [PubMed: 3019552]
2. Blum B, Bakalara N, Simpson L. A model for RNA editing in kinetoplastid mitochondria: “Guide” RNA molecules transcribed from maxicircle DNA provide the edited information. *Cell*. 1990; 60:189–198. [PubMed: 1688737]
3. Maslov DA, Simpson L. The polarity of editing within a multiple gRNA-mediated domain is due to formation of anchors for upstream gRNAs by downstream editing. *Cell*. 1992; 70:459–467. [PubMed: 1379519]
4. Gray MW. Mitochondrial evolution. *Cold Spring Harb. Perspect. Biol.* 2012; 4:a011403. [PubMed: 22952398]
5. Gray MW, Lukes J, Archibald JM, Keeling PJ, Doolittle WF. Cell biology. Irremediable complexity? *Science*. 2010; 330:920–921. [PubMed: 21071654]
6. Seiwert SD, Heidmann S, Stuart K. Direct visualization of uridylyate deletion *in vitro* suggests a mechanism for kinetoplastid RNA editing. *Cell*. 1996; 84:831–841. [PubMed: 8601307]
7. Kable ML, Seiwert SD, Heidmann S, Stuart K. RNA editing: a mechanism for gRNA-specified uridylyate insertion into precursor mRNA. *Science*. 1996; 273:1189–1195. [PubMed: 8703045]
8. Aphasizhev R, Aphasizheva I. Uridine Insertion/Deletion mRNA Editing In Trypanosomes: A Playground for RNA-guided Information Transfer. *Wiley Interdisciplinary Reviews: RNA*. 2011; 2:669–685. [PubMed: 21823228]
9. Deng J, Ernst NL, Turley S, Stuart KD, Hol WG. Structural basis for UTP specificity of RNA editing TUTases from *Trypanosoma brucei*. *EMBO J*. 2005; 24:4007–4017. [PubMed: 16281058]
10. Weng J, Aphasizheva I, Etheridge RD, Huang L, Wang X, Falick AM, Aphasizhev R. Guide RNA-Binding Complex from Mitochondria of Trypanosomatids. *Mol. Cell*. 2008; 32:198–209. [PubMed: 18951088]
11. Igo RP Jr, Lawson SD, Stuart K. RNA sequence and base pairing effects on insertion editing in *Trypanosoma brucei*. *Mol. Cell. Biol.* 2002; 22:1567–1576. [PubMed: 11839822]
12. Igo RP, Weston DS, Ernst NL, Panigrahi AK, Salavati R, Stuart K. Role of uridylylate-specific exoribonuclease activity in *Trypanosoma brucei* RNA editing. *Eukar. Cell*. 2002; 1:112–118.
13. Igo RP, Palazzo SS, Burgess ML, Panigrahi AK, Stuart K. Uridylate addition and RNA ligation contribute to the specificity of kinetoplastid insertion RNA editing. *Mol. Cell. Biol.* 2000; 20:8447–8457. [PubMed: 11046141]
14. Blanc V, Aphasizhev R, Alfonzo JD, Simpson L. The mitochondrial RNA ligase from *Leishmania tarentolae* can join RNA molecules bridged by a complementary RNA. *J. Biol. Chem.* 1999; 274:24289–24296. [PubMed: 10446205]

15. Trotter JR, Ernst NL, Carnes J, Panicucci B, Stuart K. A deletion site editing endonuclease in *Trypanosoma brucei*. *Mol. Cell.* 2005; 20:403–412. [PubMed: 16285922]
16. Carnes J, Trotter JR, Ernst NL, Steinberg A, Stuart K. An essential RNase III insertion editing endonuclease in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:16614–16619. [PubMed: 16269544]
17. Carnes J, Soares CZ, Wickham C, Stuart K. Endonuclease associations with three distinct editosomes in *Trypanosoma brucei*. *J. Biol. Chem.* 2011; 286:19320–19330. [PubMed: 21474442]
18. Carnes J, Trotter JR, Peltan A, Fleck M, Stuart K. RNA Editing in *Trypanosoma brucei* requires three different editosomes. *Mol. Cell. Biol.* 2008; 28:122–130. [PubMed: 17954557]
19. Carnes J, Schnaufer A, McDermott SM, Domingo G, Proff R, Steinberg AG, Kurtz I, Stuart K. Mutational analysis of *Trypanosoma brucei* editosome proteins KREPB4 and KREPB5 reveals domains critical for function. *RNA.* 2012; 18:1897–1909. [PubMed: 22919050]
20. Ernst NL, Panicucci B, Carnes J, Stuart K. Differential functions of two editosome exoUases in *Trypanosoma brucei*. *RNA.* 2009; 15:947–957. [PubMed: 19318463]
21. Schnaufer A, Wu M, Park YJ, Nakai T, Deng J, Proff R, Hol WG, Stuart KD. A protein-protein interaction map of trypanosome ~20S editosomes. *J. Biol. Chem.* 2010; 285:5282–5295. [PubMed: 20018860]
22. Schnaufer A, Ernst NL, Palazzo SS, O'Rear J, Salavati R, Stuart K. Separate Insertion and Deletion Subcomplexes of the *Trypanosoma brucei* RNA Editing Complex. *Mol. Cell.* 2003; 12:307–319. [PubMed: 14536071]
23. Kang X, Falick AM, Nelson RE, Gao G, Rogers K, Aphasizhev R, Simpson L. Disruption of the zinc finger motifs in the *Leishmania tarentolae* LC-4 (=TbMP63) L-complex editing protein affects the stability of the L-complex. *J. Biol. Chem.* 2004; 279:3893–3899. [PubMed: 14604987]
24. Aphasizhev R, Aphasizheva I, Simpson L. A tale of two TUTases. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:10617–10622. [PubMed: 12954983]
25. Rogers K, Gao G, Simpson L. Uridylate-specific 3' 5'-exoribonucleases involved in uridylate-deletion RNA editing in trypanosomatid mitochondria. *J. Biol. Chem.* 2007; 282:29073–29080. [PubMed: 17699520]
26. Carnes J, Lewis EN, Wickham C, Panicucci B, Stuart K. KREX2 is not essential for either procyclic or bloodstream form *Trypanosoma brucei*. *PLoS ONE.* 2012; 7:e33405. [PubMed: 22438925]
27. Kang X, Gao G, Rogers K, Falick AM, Zhou S, Simpson L. Reconstitution of full-round uridine-deletion RNA editing with three recombinant proteins. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:13944–13949. [PubMed: 16963561]
28. Aphasizhev R, Aphasizheva I, Nelson RE, Gao G, Simpson AM, Kang X, Falick AM, Sbicego S, Simpson L. Isolation of a U-insertion/deletion editing complex from *Leishmania tarentolae* mitochondria. *EMBO J.* 2003; 22:913–924. [PubMed: 12574127]
29. Ringpis GE, Aphasizheva I, Wang X, Huang L, Lathrop RH, Hatfield GW, Aphasizhev R. Mechanism of U Insertion RNA Editing in Trypanosome Mitochondria: The Bimodal TUTase Activity of the Core Complex. *J. Mol. Biol.* 2010; 399:680–695. [PubMed: 20362585]
30. Ringpis GE, Stagno J, Aphasizhev R. Mechanism of U-insertion RNA Editing in Trypanosome Mitochondria: Characterization of RET2 Functional Domains by Mutational Analysis. *J. Mol. Biol.* 2010; 399:696–706. [PubMed: 20417643]
31. Ernst NL, Panicucci B, Igo RP Jr, Panigrahi AK, Salavati R, Stuart K. TbMP57 is a 3' terminal uridylyl transferase (TUTase) of the *Trypanosoma brucei* editosome. *Mol. Cell.* 2003; 11:1525–1536. [PubMed: 12820966]
32. Kang X, Rogers K, Gao G, Falick AM, Zhou S, Simpson L. Reconstitution of uridine-deletion precleaved RNA editing with two recombinant enzymes. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:1017–1022. [PubMed: 15657144]
33. Prasad R, Beard WA, Wilson SH. Studies of gapped DNA substrate binding by mammalian DNA polymerase beta. Dependence on 5'-phosphate group. *J. Biol. Chem.* 1994; 269:18096–18101. [PubMed: 8027071]

34. Gao G, Simpson AM, Kang X, Rogers K, Nebohacova M, Li F, Simpson L. Functional complementation of *Trypanosoma brucei* RNA in vitro editing with recombinant RNA ligase. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:4712–4717. [PubMed: 15781861]
35. Schnauffer A, Panigrahi AK, Panicucci B, Igo RP, Salavati R, Stuart K. An RNA ligase essential for RNA editing and survival of the bloodstream form of *Trypanosoma brucei*. *Science*. 2001; 291:2159–2161. [PubMed: 11251122]
36. Gao G, Simpson L. Is the *Trypanosoma brucei* REL1 RNA ligase specific for U-deletion RNA editing, and is the REL2 RNA ligase specific for U-insertion editing? *J. Biol. Chem.* 2003; 278:27570–27574. [PubMed: 12748175]
37. Huang CE, Cruz-Reyes J, Zhelonkina AG, O'Hearn S, Wirtz E, Sollner-Webb B. Roles for ligases in the RNA editing complex of *Trypanosoma brucei*: band IV is needed for U-deletion and RNA repair. *EMBO J.* 2001; 20:4694–4703. [PubMed: 11532934]
38. Cruz-Reyes J, Zhelonkina AG, Huang CE, Sollner-Webb B. Distinct functions of two RNA ligases in active *Trypanosoma brucei* RNA editing complexes. *Mol. Cell. Biol.* 2002; 22:4652–4660. [PubMed: 12052873]
39. O'Hearn SF, Huang CE, Hemann M, Zhelonkina A, Sollner-Webb B. *Trypanosoma brucei* RNA editing complex: band II is structurally critical and maintains band V ligase, which is nonessential. *Mol. Cell. Biol.* 2003; 23:7909–7919. [PubMed: 14560033]
40. Ho CK, Wang LK, Lima CD, Shuman S. Structure and mechanism of RNA ligase. *Structure*. 2004; 12:327–339. [PubMed: 14962393]
41. Deng J, Schnauffer A, Salavati R, Stuart KD, Hol WG. High resolution crystal structure of a key editosome enzyme from *Trypanosoma brucei*: RNA editing ligase 1. *J. Mol. Biol.* 2004; 343:601–613. [PubMed: 15465048]
42. Ho CK, Shuman S. Bacteriophage T4 RNA ligase 2 (gp24.1) exemplifies a family of RNA ligases found in all phylogenetic domains. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:12709–12714. [PubMed: 12228725]
43. Blum B, Simpson L. Guide RNAs in kinetoplastid mitochondria have a nonencoded 3' oligo-(U) tail involved in recognition of the pre-edited region. *Cell*. 1990; 62:391–397. [PubMed: 1695552]
44. Bakalara N, Simpson AM, Simpson L. The *Leishmania* kinetoplast-mitochondrion contains terminal uridylyltransferase and RNA ligase activities. *J. Biol. Chem.* 1989; 264:18679–18686. [PubMed: 2478548]
45. Aphasizhev R, Sbicego S, Peris M, Jang SH, Aphasizheva I, Simpson AM, Rivlin A, Simpson L. Trypanosome Mitochondrial 3' Terminal Uridylyl Transferase (TUTase): The Key Enzyme in U-insertion/deletion RNA Editing. *Cell*. 2002; 108:637–648. [PubMed: 11893335]
46. Leung SS, Koslowsky DJ. Mapping contacts between gRNA and mRNA in trypanosome RNA editing. *Nucleic Acids Res.* 1999; 27:778–787. [PubMed: 9889273]
47. Leung SS, Koslowsky DJ. RNA editing in *Trypanosoma brucei*: characterization of gRNA U-tail interactions with partially edited mRNA substrates. *Nucleic Acids Res.* 2001; 29:703–709. [PubMed: 11160892]
48. Leung SS, Koslowsky DJ. Interactions of mRNAs and gRNAs involved in trypanosome mitochondrial RNA editing: Structure probing of an mRNA bound to its cognate gRNA. *RNA*. 2001; 7:1803–1816. [PubMed: 11780636]
49. Blom D, Burg J, Breek CK, Speijer D, Muijsers AO, Benne R. Cloning and characterization of two guide RNA-binding proteins from mitochondria of *Crithidia fasciculata*: gBP27, a novel protein, and gBP29, the orthologue of *Trypanosoma brucei* gBP21. *Nucleic Acids Res.* 2001; 29:2950–2962. [PubMed: 11452020]
50. Hayman ML, Read LK. *Trypanosoma brucei* RBP16 is a mitochondrial Y-box family protein with guide RNA binding activity. *J. Biol. Chem.* 1999; 274:12067–12074. [PubMed: 10207031]
51. Pelletier M, Miller MM, Read LK. RNA-binding properties of the mitochondrial Y-box protein RBP16. *Nucleic Acids Res.* 2000; 28:1266–1275. [PubMed: 10666472]
52. Vanhamme L, Perez-Morga D, Marchal C, Speijer D, Lambert L, Geuskens M, Alexandre S, Ismaïli N, Göringer U, Benne R, Pays E. *Trypanosoma brucei* TBRGG1, a mitochondrial oligo(U)-binding protein that co-localizes with an in vitro RNA editing activity. *J. Biol. Chem.* 1998; 273:21825–21833. [PubMed: 9705321]

53. Cruz-Reyes J, Zhelonkina A, Rusche L, Sollner-Webb B. Trypanosome RNA editing: simple guide RNA features enhance U deletion 100-fold. *Mol. Cell. Biol.* 2001; 21:884–892. [PubMed: 11154275]
54. Aphasizheva I, Aphasizhev R. RET1-catalyzed Uridylylation Shapes the Mitochondrial Transcriptome in *Trypanosoma brucei*. *Mol. Cell. Biol.* 2010; 30:1555–1567. [PubMed: 20086102]
55. Grams J, McManus MT, Hajduk SL. Processing of polycistronic guide RNAs is associated with RNA editing complexes in *Trypanosoma brucei*. *EMBO J.* 2000; 19:5525–5532. [PubMed: 11032819]
56. Chang HM, Triboulet R, Thornton JE, Gregory RI. A role for the Perlman syndrome exonuclease Dis3l2 in the Lin28-let-7 pathway. *Nature.* 2013; 497:244–248. [PubMed: 23594738]
57. Aphasizhev R, Aphasizheva I. Mitochondrial RNA Processing In Trypanosomes. *Res. Microbiol.* 2011; 162:655–663. [PubMed: 21596134]
58. Madina BR, Kuppan G, Vashisht AA, Liang YH, Downey KM, Wohlschlegel JA, Ji X, Sze SH, Sacchettini JC, Read LK, Cruz-Reyes J. Guide RNA biogenesis involves a novel RNase III family endoribonuclease in *Trypanosoma brucei*. *RNA.* 2011; 17:1821–1830. [PubMed: 21810935]
59. Madej MJ, Alfonzo JD, Huttenhofer A. Small ncRNA transcriptome analysis from kinetoplast mitochondria of *Leishmania tarentolae*. *Nucleic Acids Res.* 2007; 35:1544–1554. [PubMed: 17287292]
60. Madej MJ, Niemann M, Huttenhofer A, Goring HU. Identification of novel guide RNAs from the mitochondria of *Trypanosoma brucei*. *RNA Biol.* 2008; 5:84–91. [PubMed: 18418086]
61. Ochsenreiter T, Cipriano M, Hajduk SL. Alternative mRNA editing in trypanosomes is extensive and may contribute to mitochondrial protein diversity. *PLoS ONE.* 2008; 3:e1566. [PubMed: 18270563]
62. Ochsenreiter T, Hajduk SL. Alternative editing of cytochrome c oxidase III mRNA in trypanosome mitochondria generates protein diversity. *EMBO Rep.* 2006; 7:1128–1133. [PubMed: 17008930]
63. Goring HU, Koslowsky DJ, Morales TH, Stuart K. The formation of mitochondrial ribonucleoprotein complexes involving guide RNA molecules in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U.S.A.* 1994; 91:1776–1780. [PubMed: 8127880]
64. Pollard VW, Harris ME, Hajduk SL. Native mRNA editing complexes from *Trypanosoma brucei* mitochondria. *EMBO J.* 1992; 11:4429–4438. [PubMed: 1330537]
65. Peris M, Simpson AM, Grunstein J, Liliental JE, Frech GC, Simpson L. Native gel analysis of ribonucleoprotein complexes from a *Leishmania tarentolae* mitochondrial extract. *Mol. Biochem. Parasitol.* 1997; 85:9–24. [PubMed: 9108545]
66. Muller UF, Goring HU. Mechanism of the gBP21-mediated RNA/RNA annealing reaction: matchmaking and charge reduction. *Nucleic Acids Res.* 2002; 30:447–455. [PubMed: 11788706]
67. Muller UF, Lambert L, Goring HU. Annealing of RNA editing substrates facilitated by guide RNA-binding protein gBP21. *EMBO J.* 2001; 20:1394–1404. [PubMed: 11250905]
68. Lambert L, Muller UF, Souza AE, Goring HU. The involvement of gRNA-binding protein gBP21 in RNA editing-an in vitro and in vivo analysis. *Nucleic Acids Res.* 1999; 27:1429–1436. [PubMed: 10037802]
69. Allen TE, Heidmann S, Reed R, Myler PJ, Goring HU, Stuart KD. Association of guide RNA binding protein gBP21 with active RNA editing complexes in *Trypanosoma brucei*. *Mol. Cell. Biol.* 1998; 18:6014–6022. [PubMed: 9742118]
70. Koller J, Muller UF, Schmid B, Missel A, Kruft V, Stuart K, Goring HU. *Trypanosoma brucei* gBP21. An arginine-rich mitochondrial protein that binds to guide RNA with high affinity. *J. Biol. Chem.* 1997; 272:3749–3757. [PubMed: 9013632]
71. Aphasizhev R, Aphasizheva I, Nelson RE, Simpson L. A 100-kD complex of two RNA-binding proteins from mitochondria of *Leishmania tarentolae* catalyzes RNA annealing and interacts with several RNA editing components. *RNA.* 2003; 9:62–76. [PubMed: 12554877]
72. Pelletier M, Read LK. RBP16 is a multifunctional gene regulatory protein involved in editing and stabilization of specific mitochondrial mRNAs in *Trypanosoma brucei*. *RNA.* 2003; 9:457–468. [PubMed: 12649497]

73. Zikova A, Kopečna J, Schumacher MA, Stuart K, Trantirek L, Lukes J. Structure and function of the native and recombinant mitochondrial MRP1/MRP2 complex from *Trypanosoma brucei*. *Int. J. Parasitol.* 2008; 38:901–12. [PubMed: 18295767]
74. Hashimi H, Zikova A, Panigrahi AK, Stuart KD, Lukes J. TbRGG1, an essential protein involved in kinetoplastid RNA metabolism that is associated with a novel multiprotein complex. *RNA.* 2008; 14:970–980. [PubMed: 18369185]
75. Hashimi H, Cicova Z, Novotna L, Wen YZ, Lukes J. Kinetoplastid guide RNA biogenesis is dependent on subunits of the mitochondrial RNA binding complex 1 and mitochondrial RNA polymerase. *RNA.* 2009; 15:588–599. [PubMed: 19228586]
76. Panigrahi AK, Zikova A, Dalley RA, Acestor N, Ogata Y, Anupama A, Myler PJ, Stuart KD. Mitochondrial complexes in *trypanosoma brucei*: a novel complex and a unique oxidoreductase complex. *Mol. Cell Proteomics.* 2007; 7:534–545. [PubMed: 18073385]
77. Hashimi H, Zimmer SL, Ammerman ML, Read LK, Lukes J. Dual core processing: MRB1 is an emerging kinetoplast RNA editing complex. *Trends Parasitol.* 2013; 29:91–99. [PubMed: 23305619]
78. Etheridge RD, Aphasizheva I, Gershon PD, Aphasizhev R. 3' adenylation determines mRNA abundance and monitors completion of RNA editing in *T. brucei* mitochondria. *EMBO J.* 2008; 27:1596–1608. [PubMed: 18464794]
79. Aphasizheva I, Maslov D, Wang X, Huang L, Aphasizhev R. Pentatricopeptide Repeat Proteins Stimulate mRNA Adenylation/Uridylation to Activate Mitochondrial Translation in *Trypanosomes*. *Mol. Cell.* 2011; 42:106–117. [PubMed: 21474072]
80. Ammerman ML, Presnyak V, Fisk JC, Foda BM, Read LK. TbRGG2 facilitates kinetoplastid RNA editing initiation and progression past intrinsic pause sites. *RNA.* 2010; 16:2239–2251. [PubMed: 20855539]
81. Ammerman ML, Downey KM, Hashimi H, Fisk JC, Tomasello DL, Faktorova D, Kafkova L, King T, Lukes J, Read LK. Architecture of the trypanosome RNA editing accessory complex. MRB1, *Nucleic Acids Res.* 2012; 40:5637–5650. [PubMed: 22396527]
82. Kafkova L, Ammerman ML, Faktorova D, Fisk JC, Zimmer SL, Sobotka R, Read LK, Lukes J, Hashimi H. Functional characterization of two paralogs that are novel RNA binding proteins influencing mitochondrial transcripts of *Trypanosoma brucei*. *RNA.* 2012; 18:1846–1861. [PubMed: 22898985]
83. Ammerman ML, Hashimi H, Novotna L, Cicova Z, McEvoy SM, Lukes J, Read LK. MRB3010 is a core component of the MRB1 complex that facilitates an early step of the kinetoplastid RNA editing process. *RNA.* 2011; 17:865–877. [PubMed: 21451155]
84. Missel A, Souza AE, Norskau G, Goring HU. Disruption of a gene encoding a novel mitochondrial DEAD-box protein in *Trypanosoma brucei* affects edited mRNAs. *Mol. Cell. Biol.* 1997; 17:4895–4903. [PubMed: 9271369]
85. Simpson L, Aphasizhev R, Lukes J, Cruz-Reyes J. Guide to the nomenclature of kinetoplastid RNA editing: a proposal. *Protist.* 2010; 161:2–6. [PubMed: 19945343]
86. Hernandez A, Madina BR, Ro K, Wohlschlegel JA, Willard B, Kinter MT, Cruz-Reyes J. REH2 RNA helicase in kinetoplastid mitochondria: ribonucleoprotein complexes and essential motifs for unwinding and guide RNA (gRNA) binding. *J. Biol. Chem.* 2010; 285:1220–1228. [PubMed: 19850921]
87. Panigrahi AK, Allen TE, Stuart K, Haynes PA, Gygi SP. Mass spectrometric analysis of the editosome and other multiprotein complexes in *Trypanosoma brucei*. *J. Am. Soc. Mass Spectrom.* 2003; 14:728–735. [PubMed: 12837594]
88. Li F, Herrera J, Zhou S, Maslov DA, Simpson L. Trypanosome REH1 is an RNA helicase involved with the 3'-5' polarity of multiple gRNA-guided uridine insertion/deletion RNA editing. *Proc. Natl. Acad. Sci. U.S.A.* 2011; 108:3542–3547. [PubMed: 21321231]
89. Bhat GJ, Myler PJ, Stuart K. The two ATPase 6 mRNAs of *Leishmania tarentolae* differ at their 3' ends. *Mol. Biochem. Parasitol.* 1991; 48:139–150. [PubMed: 1837067]
90. Bhat GJ, Koslowsky DJ, Feagin JE, Smiley BL, Stuart K. An extensively edited mitochondrial transcript in kinetoplastids encodes a protein homologous to ATPase subunit 6. *Cell.* 1990; 61:885–894. [PubMed: 2140530]

91. Read LK, Stankey KA, Fish WR, Muthiani AM, Stuart K. Developmental regulation of RNA editing and polyadenylation in four life cycle stages of *Trypanosoma congolense*. *Mol. Biochem. Parasitol.* 1994; 68:297–306. [PubMed: 7739675]
92. Kao CY, Read LK. Opposing effects of polyadenylation on the stability of edited and unedited mitochondrial RNAs in *Trypanosoma brucei*. *Mol. Cell. Biol.* 2005; 25:1634–1644. [PubMed: 15713623]
93. Militello KT, Read LK. UTP-dependent and -independent pathways of mRNA turnover in *Trypanosoma brucei* mitochondria. *Mol. Cell. Biol.* 2000; 20:2308–2316. [PubMed: 10713154]
94. Ryan CM, Read LK. UTP-dependent turnover of *Trypanosoma brucei* mitochondrial mRNA requires UTP polymerization and involves the RET1 TUTase. *RNA.* 2005; 11:763–773. [PubMed: 15811918]
95. Small ID, Peeters N. The PPR motif - a TPR-related motif prevalent in plant organellar proteins. *Trends Biochem. Sci.* 2000; 25:46–47. [PubMed: 10664580]
96. Schmitz-Linneweber C, Small I. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. *Trends Plant Sci.* 2008; 13:663–670. [PubMed: 19004664]
97. Zikova A, Panigrahi AK, Dalley RA, Acestor N, Anupama A, Ogata Y, Myler PJ, Stuart K. *Trypanosoma brucei* mitochondrial ribosomes: affinity purification and component identification by mass spectrometry. *Mol. Cell Proteomics.* 2008; 7:1286–1296. [PubMed: 18364347]
98. Aphasizhev R, Aphasizheva I. Emerging roles of PPR proteins in trypanosomes: Switches, blocks, and triggers. *RNA Biol.* 2013; 10 in press.
99. Pusnik M, Small I, Read LK, Fabbro T, Schneider A. Pentatricopeptide repeat proteins in *Trypanosoma brucei* function in mitochondrial ribosomes. *Mol. Cell. Biol.* 2007; 27:6876–6888. [PubMed: 17646387]
100. Ridlon L, Skodova I, Pan S, Lukes J, Maslov DA. The Importance of the 45S Ribosomal Small Subunit-related Complex for Mitochondrial Translation in *Trypanosoma brucei*. *J. Biol. Chem.* 2013; 288:32963–32978. [PubMed: 24089529]
101. Aphasizheva I, Maslov DA, Aphasizhev R. Kinetoplast DNA-encoded ribosomal protein S12: a possible functional link between mitochondrial RNA editing and translation in *Trypanosoma brucei*. *RNA Biol.* 2013 in press.
102. Stuart KD, Schnauffer A, Ernst NL, Panigrahi AK. Complex management: RNA editing in trypanosomes. *Trends Biochem. Sci.* 2005; 30:97–105. [PubMed: 15691655]

Highlights

- RNA editing is essential for mitochondrial genome expression in trypanosomes
- Specific patterns of U-insertions and U-deletions are directed by guide RNAs
- Editing reactions are catalyzed by the RNA editing core complex
- Guide RNA binding and other axillary complexes participate in the editing pathway
- Polyadenylation stabilizes edited mRNAs and enables their binding to the ribosome

Figure 1.

The RNA editing core complex catalyzes elementary RNA editing reactions. Direct protein-protein interactions within core complex are depicted by black bars. Roman numerals signify three elementary steps of RNA editing: mRNA cleavage, U-deletion or insertion and mRNA ligation. MP: mitochondrial protein (structural and/or RNA binding components); REX: RNA editing exonuclease; REN: RNA editing endonuclease; REL: RNA editing ligase; RET: RNA editing TUTase; anchor: 5-15-nt long double-stranded region formed by the 5' portion of the gRNA and pre-edited mRNA.

Table 1Components of the RNA editing core complex from *T. brucei*.

Alternative names for proteins	Gene ID	Proposed function
MP81/KREPA1	Tb927.2.2470	structural, U-insertion subdomain organizer
MP63/KREPA2	Tb927.10.8210	structural, U-deletion subdomain organizer
MP42/KREPA3	Tb927.8.620	structural
MP24/KREPA4	Tb927.10.5110	structural, RNA binding
MP19/KREPA5	Tb927.8.680	structural
MP18/KREPA6	Tb927.10.5120	structural, RNA binding
REN1/KREN1	Tb927.1.1690	insertion site specific endonuclease
REN2/KREN2	Tb927.10.5440	deletion site specific endonuclease
REN3/KREN3	Tb927.10.5320	cis-editing site specific endonuclease
MP46/KREPB4	Tb927.11.2990	structural, heterodimer with endonuclease
MP44/KREPB5	Tb927.11.940	structural, endonuclease
MP49/KREPB6	Tb927.3.3990	structural, part of KREN3 module
MP47/KREPB7	Tb927.9.5630	structural, part of KREN2 module
MP41/KREPB8	Tb927.9.5630	structural, part of KREN1 module
REX1/KREX1	Tb927.7.1070	U-specific exonuclease
REX2/KREX2	Tb927.10.3570	U-specific exonuclease
REL1/KREL1	Tb927.9.4360	RNA ligase
REL2/KREL2	Tb927.1.3030	RNA ligase
RET2/KRET2	Tb927.7.1550	TUTase

Two alternative nomenclatures are used in the current literature to describe RECC subunits [85;102]. Gene identification numbers are provided according to TriTrypDB 5.0 database release (<http://tritrypdb.org/tritrypdb/>).