Complex management: RNA editing in trypanosomes

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Most mitochondrial mRNAs in kinetoplastids require editing, that is, the posttranscriptional insertion and deletion of uridine nucleotides that are specified by guide RNAs and catalyzed by multiprotein complexes. Recent studies have identified many of the proteins in these complexes, in addition to some of their functions and interactions. Although much remains unknown, a picture of highly organized complexes is emerging that shows that the complex that catalyzes the central steps of editing is partitioned into distinct insertion and deletion editing subcomplexes. These subcomplexes coordinate hundreds of ordered catalytic steps that function to produce a single mature mRNA. The dynamic processes, which might entail interactions among multiprotein complexes and changes in their composition and conformation, remain to be elucidated.

Introduction

Most trypanosomatid mitochondrial mRNAs undergo RNA editing by which precursor mRNA (pre-mRNA) sequences are changed, often extensively, by the insertion and less frequently the deletion of uridine nucleotides (Us). The edited mRNAs are translated into components of the oxidative phosphorylation system including subunits of respiratory complexes I (NADH–ubiquinone oxidoreductase), III (cytochrome bc_1), IV (cytochrome oxidase) and V (ATP synthase). The pre-edited mRNAs are encoded in a larger mitochondrial DNA, termed the 'maxicircle', whereas smaller mitochondrial DNAs or 'minicircles' encode guide RNAs (gRNAs) that specify the editing. *Trypanosoma brucei* has roughly 50 identical 22-kb maxicircles and ∼10 000 heterogeneous 1-kb minicircles, each of which encodes three or four gRNAs, constituting a total of >1200 different gRNAs. The maxicircles of different trypanosomatid species encode the same mRNAs (and rRNAs) but differ in which RNAs are edited and to what extent. The general mechanism of editing has been determined (Figure 1).

Editing is catalyzed by multiprotein complexes that have not yet been fully defined or characterized. Several laboratories have purified a complex that sediments at ∼20 Svedberg (20S) on glycerol gradients, contains the four key enzyme activities and catalyzes *in vitro* editing. We call this multicatalyst complex the '20S editosome'

here for simplicity. Larger, less-defined complexes (∼40S) also contain these activities in addition to gRNAs and edited mRNAs, which, coupled with their association with other proteins or complexes such as those that add oligo(U) tails to gRNA or transport RNAs to the editosome, could account for the larger complex size. Editosomes must be dynamic during editing because of their interactions with other molecules and complexes and their molecular movement associated with catalysis, RNA translocation and gRNA displacement, which might even entail compositional changes.

Numerous proteins have been identified in editing complexes that have been purified from *T. brucei* and *Leishmania tarentolae* by various methods [1–7] and orthologs of these proteins have been identified in the *Trypanosoma cruzi* and *Leishmania major* databases [8] (Box 1). These proteins are related in pairs or sets by sequence similarities that, as we describe below, reflect the functions of some of these proteins and the structural and functional division of the editosome into insertion and deletion subcomplexes (Figure 2). Here, we describe what is currently known about the composition, organization and functions of the multi-protein complexes that are involved in RNA editing.

Endonuclease

Editing starts with endonucleolytic cleavage of pre-mRNA at a cleavage site determined by the interaction between a gRNA and its cognate mRNA. The 5′ region of a gRNA can form an 'anchor' duplex with its cognate mRNA 3′ to the region to be edited. Hundreds of different gRNA

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> **Box 1. Nomenclature**
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> In this review, we have introduced a new nomenclature that is intended to be independent of species and more consistent than the previous ones. Kinetoplastid RNA editing (KRE) proteins with no known function are designated with a P plus an A, B or C to indicate groups with sequence and/or motif similarity, and are numbered, generally in descending order of size. Proteins with experimentally demonstrated functions are designated by a single letter appended to KRE, L for RNA ligase, T for 3′ terminal uridylyl transferase, and H for RNA helicase, and are numbered by size when there is more than one. We propose to use N for RNA endonuclease and X for RNA exonuclease when the proteins responsible for these activities are identified. Previous nomenclatures for the editing complex proteins were based either on molecular weight (e.g. MP81, mitochondrial protein of 81 kDa in *T. brucei*) or on the order of bands obtained in an SDS–PAGE fractionation of a purified complex (e.g. the LC-X and band X nomenclature in *Leishmania* and *T. brucei*, respectively).
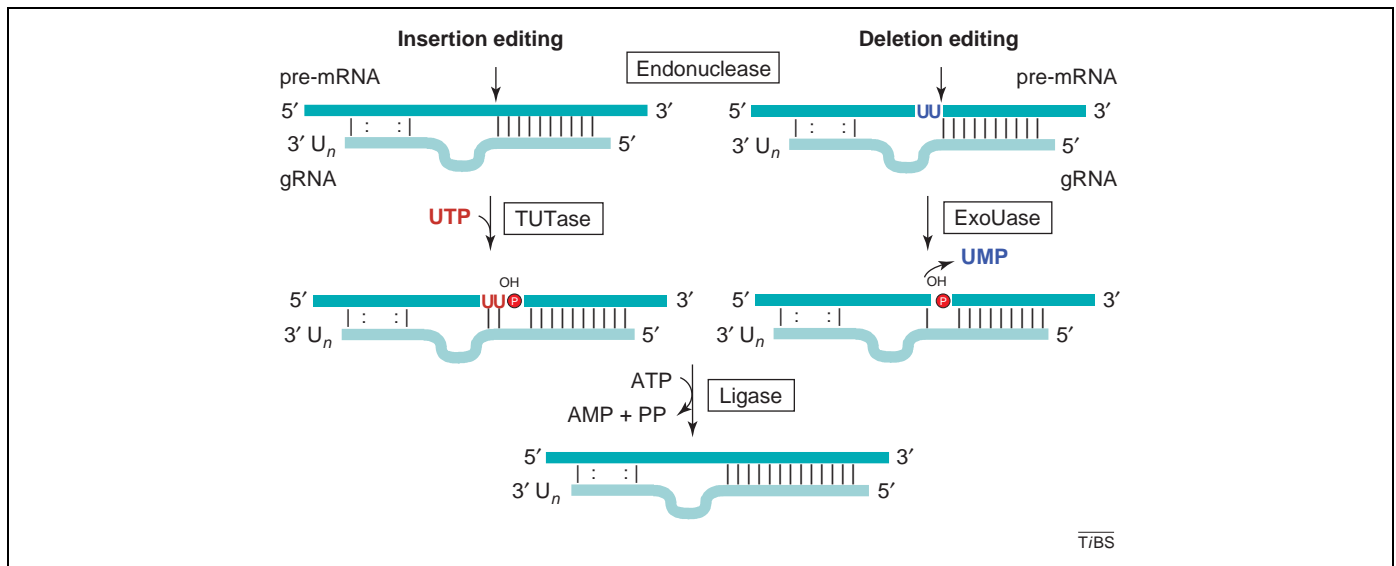
Figure 1. General mechanism of RNA editing. Shown are the catalytic events in insertion and deletion editing. pre-mRNAs (dark blue strands) are edited progressively 3′ to 5′ with each gRNA (light blue strands) specifying the editing of several sites. Interaction between the RNAs by Watson–Crick base-pairs (unbroken lines) and G•U base-pairs (colons) determines the sites of cleavage and number of U nucleotides that are added or removed. The gRNAs have 3′ oligo(U) tails that are added posttranscriptionally and are essential for editing, perhaps by facilitating interactions with pre-mRNA 5′ to the editing site. Editing occurs by a series of coordinated catalytic steps. Endonucleolytic cleavage of the pre-mRNA by an endonuclease occurs upstream of the anchor duplex (8–10 bp) between the pre-mRNA and its 'cognate' gRNA (arrow). Us are either added to the 5′ cleavage fragment by a TUTase in insertion editing or removed by an ExoUase in deletion editing, as specified by the sequence of the gRNA. The resultant 5′ and 3′ mRNA fragments are then ligated by an RNA ligase. Several cycles of coordinated catalytic steps occur until all of the sites specified by a gRNA are edited, resulting in complementarity (G•U, A•U and G•C base-pairing) between the edited mRNA and the gRNA, except at the gRNA termini. Editing by each gRNA creates a sequence that is complementary to the anchor region of the subsequent gRNA to be used, thereby enabling the sequential use of the multiple gRNAs that are required to edit the mRNAs in full.

sequences, when paired with their cognate mRNAs, present diverse nucleotide sequences to the editosome endonuclease (or endonucleases). This implies that recognition of the cleavage (editing) site by the endonuclease is complicated.

Cleavage of the pre-mRNA *in vitro* typically occurs at an unpaired nucleotide immediately upstream of the gRNA–mRNA anchor duplex, leaving the phosphate on the 3′ cleavage product [9–11]. The anchor duplex alone, however, is not invariably sufficient to provide the specificity for endonucleolytic cleavage [12]. Thus, as suggested by structural mapping studies [13], structural features of the interacting pre-mRNA–gRNA pair might provide the basis for recognition by endonucleases.

Mitochondrial extracts from *T. brucei* and *L. tarentolae* have several endonuclease activities [11,14–16]. Endonucleolytic cleavage during *in vitro* deletion editing requires, and is enhanced by, adenosine nucleotides; by contrast, cleavage at insertion sites is inhibited by increasing concentrations of adenosine nucleotides [17], implying that distinct editing endonuclease activities are involved. The editosome endonucleases have not been identified but seven proteins with nuclease motifs, termed KREPC1, KREPC2 and KREPB1–KREPB5 (previously called *Tb*MP100, *Tb*MP99, *Tb*MP90, *Tb*MP67, *Tb*MP61, *Tb*MP46 and *Tb*MP44), have been identified in editosomes purified from *T. brucei* and *L. tarentolae* [3,4,7], and orthologs of these proteins are present in *L. major* and *T. cruzi* [8].

KREPC1 and KREPC2 have sequence similarity and each contain an N-terminal 5′→3′ exonuclease and a C-terminal endonuclease/exonuclease/phosphatase (EEP) domain [8]. KREPC2 is likely to be an editosome U-specific

3′→5′ exonuclease (ExoUase [18]; see below), but one or both of these proteins might have endonucleolytic activity. The other five proteins each contain a N-terminal U1-like zinc-finger domain and share varying degrees of sequence similarity, primarily in their RNase-III-like region [8]. All orthologs of KREPB1, KREPB2 and KREPB3 have conserved signature amino acid residues that are required for catalysis in their RNase III motifs and a C-terminal motif for binding double-stranded RNA (dsRNA). In the KREPB4 and KREPB5 orthologs, the RNase III motif is less conserved and lacks at least two of the signature amino acids; in addition, each of these proteins has a C-terminal Pumilio RNA-binding domain rather than a dsRNA-binding motif [8].

The conservation of the RNase III motif in KREPB1, KREPB2 and KREPB3 makes these proteins likely candidates for the editosome endonucleases, because many RNase III proteins process RNA by dsRNA cleavage [3,8,19]. Their U1-like zinc-finger and dsRNA-binding domains might function in interactions with editosome proteins and substrate RNAs. The divergence in the RNase III motif in KREPB4 and KREPB5, coupled with the disruption of the editosome that occurs on knockdown of KREPB5 expression [20], suggests that these two proteins might function in molecular interaction rather than in catalysis. Knockdown of the expression of KREPB5 is lethal in the bloodstream form of *T. brucei*, as it is for all genes so far tested that are normally required for editing (see later).

The several potential editosome nucleases might reflect functional division of the complex into insertion and deletion subcomplexes (see below), could account for the several endonuclease activities detected (see above), and
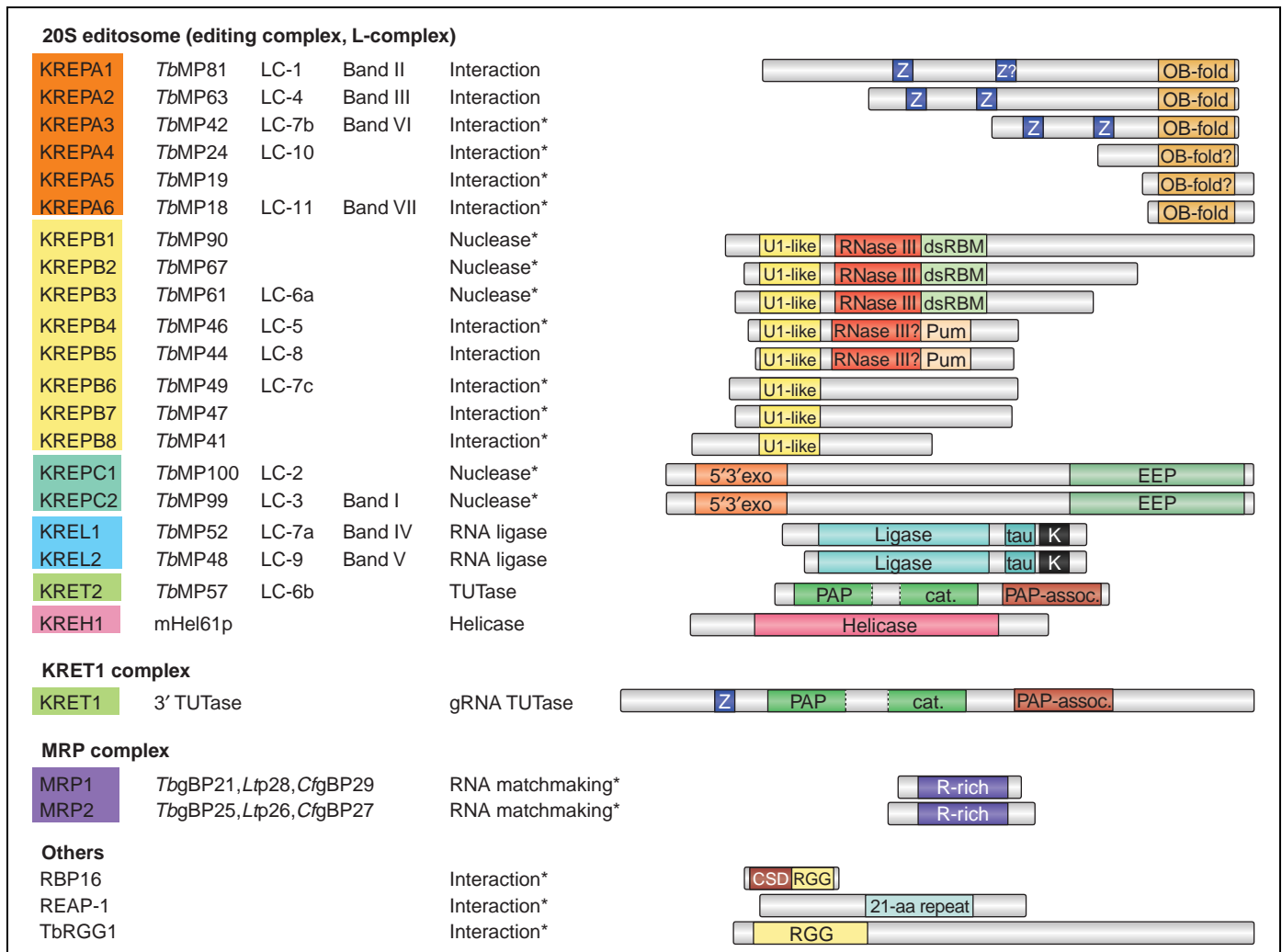
Figure 2. RNA editing complexes and their components. Many of the proteins involved in RNA editing are related in pairs or sets by sequence similarities that reflect the functions of these proteins. Roles have been demonstrated by expression knockdown studies or by the stable association of the proteins with complexes that have roles in editing. 'Interaction' refers to RNA and/or protein binding in the absence of any known catalytic activity. Asterisks indicate roles that have not been verified experimentally. KREPA1–KREPA6 (previous protein names are indicated to the right of each protein; see Box 1) are related and each has a putative OB-fold domain. Some of these proteins contain C2H2-type zinc-finger domains (Z) and function in specific molecular interactions [1,18]. The editosome endonucleases have not been identified, but five of the editosome proteins, KREPB1–KREPB5, that have low sequence similarity and RNase III or RNase-III-like motifs in combination with either dsRNA binding (dsRBM) or Pumilio (Pum) domains are candidate endonucleases [3]. The functions of the related proteins KREPB6, KREPB7 and KREPB8, which share a U1-like zinc-finger domain (U1-like) with KREPB1–KREPB5, are unknown. KREPC2 might be an ExoUase [18], but the function of its relative, KREPC1, has not been determined. Both proteins contain 5′→3′ exoribonuclease (5′3′exo) and endonuclease/exonuclease/phosphatase (EEP) domains. The two related RNA ligases KREL1 and KREL2 contain ligase signature motifs in their N-terminal regions and putative microtubule-associated tau and kinesin light chain (K) domains in their C-terminal regions [6,8,32,33]. KRET2 is the 3′ TUTase of the 20S editosome; the related TUTase KRET1 does not purify with 20S complexes but is present in complexes that catalyze addition of the 3′ oligo(U) tail to gRNAs [27,28,30]. Both proteins have PAP catalytic (PAP-cat.) domains that have a large insertion between conserved amino acids and PAP-associated (PAP-assoc.) domains. KREH1 is an editosome RNA helicase [52]. MRP1 and MRP2 are also related, contain an arginine-rich (R-rich) domain and have been identified in a separate complex that might be involved in RNA annealing [60]. Other proteins that bind gRNA or mRNA but do not seem to be associated stably with the 20S editosome are RBP16, which contains cold-shock (CSD) and RGG RNA-binding (RGG) domains [66], REAP-1 [69] and TbRGG1, which also has an RGG domain [70]. The question mark indicates weak or potentially disrupted domains or motifs.

could provide the basis for the recognition of diverse editing sites.

Exonuclease

The ExoUase in *T. brucei* removes non-base-paired U nucleotides after cleavage of deletion editing sites [12,21], and deletion editing is enhanced by an increase in base-pairing potential upstream of the editing site [22]. A U-specific exonuclease has been partially purified from *L. tarentolae* [23].

KREPC2 (*Tb*MP99) is probably an editosome ExoUase because a KREL1 tandem affinity purification (TAP)-tagged subcomplex consisting of it, KREL1 and KREPA2

(*Tb*MP63) catalyzes accurate U removal and ligation (i.e. pre-cleaved deletion editing) [18]. KREPC2 has an N-terminal $5′→3′$ exonuclease motif and a C-terminal EEP motif that has exonucleolytic and endonucleolytic activities in many proteins [8]. Orthologs of KREPC2 have been identified in *T. cruzi*, *L. tarentolae* and *L. major*, although the *Leishmania* orthologs are smaller and lack the EEP domain [4,8], the significance of which is unknown.

KREPC1 (*Tb*MP100) is related to KREPC2, especially in the EEP domain, and might also be an ExoUase [3]. The presence of two domains in these proteins implies that KREPC1 and KREPC2 might be multifunctional in *T. brucei* and the two related proteins might have

complementary functions to accommodate different substrates or stages of the life cycle.

TUTase

In insertion editing, Us are added to the 3′ end of the 5′ pre-mRNA fragment by a terminal uridylyl transferase (TUTase) as specified by gRNA. Addition of U is enhanced by an upstream base-pair and by the base-pairing of the added Us with gRNA purines (which increases subsequent ligation), but is biased against a pre-mRNA pyrimidine immediately 5′ to the editing site (in keeping with the few C and no U nucleotides observed at this position *in vivo*) [24,25].

The editosome TUTase KRET2 was identified in purified *T. brucei* and *L. tarentolae* editosomes and contains a nucleotidyl transferase domain and poly(A) polymerase (PAP) core and associated domains [3,4,26]. Another TUTase, KRET1, is related to KRET2, contains the nucleotidyl transferase and PAP domains, and is approximately twice the size of KRET2 with an N-terminal C2H2 zinc-finger that is essential for its catalytic activity [27,28]. On the basis of their nucleotidyl transferase catalytic signature, the TUTases are members of the DNA polymerase-β superfamily but they have a unique large insertion between the conserved aspartate residues of this superfamily [4,8].

Whereas KRET2 adds the number of Us specified by the gRNA to pre-cleaved insertion editing substrates, KRET1 does not add U to dsRNA [26]. KRET2 preferentially adds a U to RNAs with an A- or G-terminal nucleotide, which matches the purine bias at this position in natural editing sites [24]. KRET2 adds one U to a single-stranded RNA, whereas KRET1 adds hundreds of Us to single-stranded RNAs without a 3′ terminal nucleotide preference [26,27]. KREPA1 (*Tb*MP81) interacts with KRET2 *in vivo* and specifically stimulates the TUTase activity of KRET2 *in vitro* [18,26].

Knockdown of KRET2 expression by RNA interference (RNAi) inhibits trypanosome growth, reduces edited RNA abundance *in vivo*, and results in specific loss of *in vitro* insertion editing, indicating that KRET2 functions as the TUTase that adds Us in insertion editing [29]. Knockdown of KRET1 expression by RNAi also results in inhibition of trypanosome growth and a reduction in edited RNAs, indicating that it has a role in editing; however, it does not effect *in vitro* pre-cleaved insertion editing. Knockdown of KRET1 expression results in an accumulation of gRNAs with shorter oligo(U) tails, whereas knockdown of KRET2 expression has no effect on the length of the gRNA oligo(U) tail [29]. In addition, a significant portion of the cellular gRNAs co-immunoprecipitate with KRET1 and gRNA has been shown to interact with KRET1 by UV crosslinking [27]. These data indicate that KRET1 adds the oligo(U) tail to gRNA.

Thus, KRET1 and KRET2 have essential but different roles in editing. The KRET1 knockdown experiments indicate that the gRNA oligo(U) tail is essential *in vivo*, although it is not essential for editing *in vitro* [29,30]. It might stabilize the interaction of the 3′ region of the gRNA with the 5′ cleavage product of mRNA, as implied by the enhanced *in vitro* editing that results from increased base-pairing upstream of the editing site [25].

Ligase

The two RNA ligases in the 20S editosome complex, KREL1 and KREL2, belong to the superfamily of covalent nucleotidyl transferases (which includes RNA capping enzymes and DNA and RNA ligases) [2,6,31–33]. Their closest relative is T4 phage RNA ligase 2 [34]. The reaction pathway involves covalent binding of AMP by a lysine residue (via a phosphoamide linkage) and requires cleavage of ATP between its α- and β-phosphates [35–37]. The natural editing ligase substrates are essentially nicked dsRNAs that are completely base-paired after the correct addition or removal of U nucleotides. Indeed, editosome ligases prefer such substrates to those with gaps or overhangs [10,12,21,37–40], which probably contributes to the accuracy of editing.

The two editosome RNA ligases are similar (41% identity), although there is greater similarity between respective orthologs among trypanosomatids, indicating that a gene duplication event preceded the divergence of the trypanosomatid species around 10^8 years ago [8,41]. Both ligases contain five signature motifs that are conserved in all covalent nucleotidyl transferases (Figure 2), and sequence alignments with other members of this superfamily have identified Lys87 and Lys57 in KREL1 and KREL2, respectively, as the lysines responsible for the covalent binding of AMP (in the first step of the ligation pathway) [32,33]. The C termini of the RNA ligases lack the oligonucleotide-binding (OB)-fold domains that are present in the C termini of DNA ligases and RNA capping enzymes, in addition to various other proteins that bind to single- or double-stranded nucleic acids [18,34,42,43]. In DNA ligases, OB-fold domains are important for substrate specificity and strand joining [42,44]. Notably, OB-fold domains have been predicted to be present in KREPA2 and KREPA1 (Figure 2), two other components of the 20S editosome that directly interact with KREL1 and KREL2, respectively; thus, it has been suggested that these partner proteins provide the OB-fold domains *in trans* [18].

KREL1 is required for RNA editing as shown by both knockdown of its expression and overexpression of a mutated ectopic allele that abolishes enzyme function [33,45,46]. By contrast, knockdown of KREL2 has no effect on RNA editing [45,47,48]. This observation suggests either that KREL1 can compensate for the loss of KREL2 or that KREL2 has no function in RNA editing *in vivo*. The latter seems unlikely because the following evidence suggests that KREL1 and KREL2 are associated with the deletion and insertion types of RNA editing, respectively.

First, overexpression of mutationally inactivated KREL1 in procyclic *T. brucei* has been shown specifically to affect deletion editing [46], although this finding has not been reproduced by others [45]. Second, ATP and pyrophosphate differentially affect the KREL1 and KREL2 ligases and have corresponding effects on *in vitro* deletion and insertion editing assays [38]. Third, knockdown of KREPA1, a protein that specifically associates with KREL2 (see below), leads to loss of KREL2 but not

KREL1 from the editosome, and preferentially inhibits insertion editing [47]. Last, studies using a combination of TAP, yeast two-hybrid analysis, and co-immunoprecipitation have identified two subcomplexes: one containing KREL1 that can perform the ExoUase and RNA ligase steps of deletion editing *in vitro*, and another containing KREL2 that can perform the U addition and ligation steps of insertion editing *in vitro* [18].

A null mutant of KREL2 has not been reported and thus it cannot be excluded that low levels of KREL2 protein present in the knockdown experiments might be sufficient to support editing. Indeed, knockdown of KREL2 results in morphological changes in trypanosomes, although their growth rate is not affected [45,47,48]. Conceivably, it is possible that KREL2 functions in insertion editing *in vivo*, but that KREL1 can also fulfill this function because KREL1, unlike KREL2, is tolerant of gaps and overhangs [18,38,49]. KREL1 has been also proposed to be required for RNA repair [46] arising from misguiding by non-cognate gRNAs [50]. Recently, the structure of the catalytic domain of KREL1 has been solved, representing the first crystal structure obtained for an editosome protein [51] (Figure 3).

Helicase

Several gRNAs are used to edit most pre-mRNAs in full and each must be displaced, perhaps by an RNA helicase, at least from the sequence that it creates to enable binding by the subsequent gRNA and possibly also from the mRNA completely before translation. 20S editosomes purified by

Figure 3. ATP-binding pocket of KREL1. The crystal structure of the KREL1 catalytic domain (shown with the protein surface in green) was obtained in a complex with ATP (shown in ball-and-stick notation, with C in yellow, N in dark blue, O in red, and P in light blue) and Mg^{2+} (gray sphere) and apparently captured the enzyme before step 1 of the pathway, providing insight into its catalytic mechanism [51]. ATP is coordinated via its β- and γ-phosphates to a single Mg^{2+} ion and is surrounded by conserved residues from all five signature motifs (labeled in yellow). A second Mg^{2+} ion would coordinate the ATP α-phosphate and promote adenylation of the ligase at Lys87, according to the two-metal-ion mechanism of RNA ligases [79]. Another distinct feature of the ATP-binding pocket of KREL1 is the presence of three water molecules at the deep end of the pocket (not visible) that might provide opportunities for structure-based drug design. Not shown in this close-up of the ATP-binding pocket are loop regions that might be involved in substrate recognition and/or protein–protein interaction [51]. Image generated with PyMOL (http://www.pymol.org).

biochemical and immunoaffinity methods contain the DEAD box helicase KREH1 (previously called mHel61p) [52], although this helicase has not been found in editosomes purified using TAP tags on seven different editosome proteins [3].

KREH1-null mutants of the procyclic form of *T. brucei* are viable but grow slowly and show partial inhibition of editing, suggesting that the gene encoding KREH1 is not essential [52]. Database searches have identified another putative mitochondrial helicase [53], however, which might compensate for KREH1 function. Thus, the specific functions of helicases in editing and the nature of their association with the editosome are unclear.

Other 20S editosome proteins

The 20S editosome also contains several other proteins with no predicted catalytic function. Six of these proteins, termed KREPA1–KREPA6 (*Tb*MP81, *Tb*MP63, *Tb*MP42, *Tb*MP24, *Tb*MP19 and *Tb*MP18), and their orthologs in *L. major*, and *T. cruzi* share varying degrees of sequence similarity [1,8]. KREPA1, KREPA2 and KREPA3 have two conserved C2H2 zinc-finger domains, although the C-terminal zinc-finger domain in KREPA1 contains additional amino acids. All six proteins have conserved C-terminal sequences that resemble an OB-fold motif [8,18] (Figure 2). These features suggest that these proteins function in RNA–protein and protein–protein interactions.

RNAi knockdown of KREPA1 expression inhibits *T. brucei* growth and results in a loss of KREL2 and insertion editing [47,48]. Similarly, RNAi knockdown of KREPA2 results in KREL1 loss and blocks cell growth and *in vitro* editing [54]. Both knockdowns also result in reduced endonucleolytic activity associated with RNA editing [47,54]. Taken together, these studies indicate that members of this family of proteins are essential for RNA editing and function in protein interactions that are crucial to editosome integrity, including the association and activity of catalytic proteins of the editosome.

Three other proteins, KREPB6, KREPB7 and KREPB8 (*Tb*MP49, *Tb*MP47 and *Tb*MP41), share varying degrees of sequence similarities and have U1-like zinc-finger motifs, suggesting that they have roles in molecular interaction.

Structure and organization of the 20S editosome

The 20S editosome complex identified in different laboratories is the smallest native particle to be isolated so far that can perform a full round of *in vitro* editing; thus, it seems to represent a catalytic core complex [1–5,7]. The architecture of this 20S editosome and the functional interactions among its components that seem to partition and to coordinate the activities of the enzymes in catalyzing the steps of RNA editing are beginning to emerge (Figure 4).

Combined gene inactivation, gene knock-in, yeast two-hybrid, biochemical and co-immunoprecipitation studies indicate that the editing activities and structure of the 20S editosome are highly integrated. Both TUTase and ExoUase are severely inhibited if the 5′ monophosphate is absent from the 3′ RNA cleavage fragment or if the ligase activity is inhibited [21,40]. Knockdown of KREPA1 or KREPA2 expression leads not only to the respective loss of
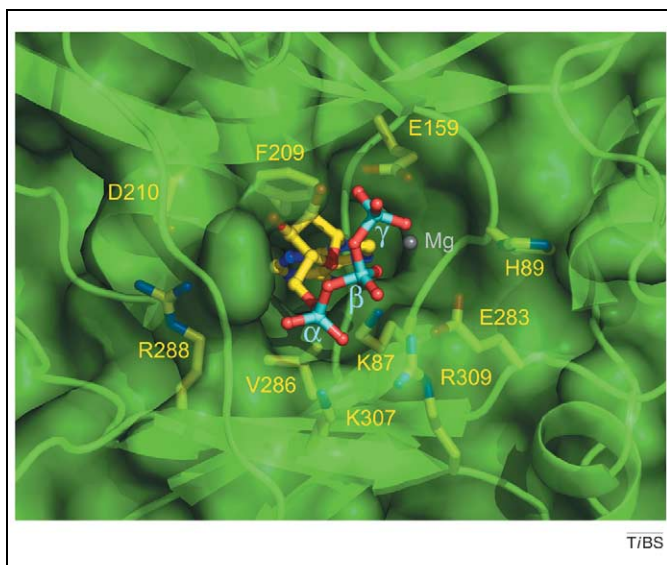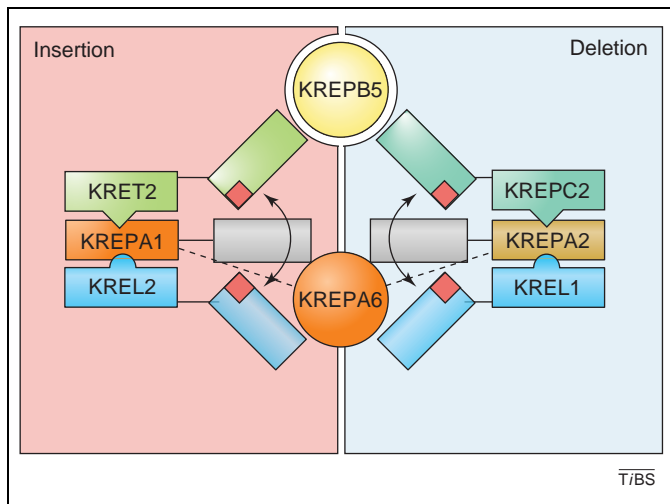
Figure 4. Subcomplexes and protein–protein interactions in the 20S editosome. Insertion and deletion editing are partitioned into editosome subcomplexes [4,18], although perhaps not exclusively, and it has been speculated that the structural organization of the subcomplexes provides the order in which the catalytic steps of editing occur [18]. The two zinc-finger proteins KREPA1 and KREPA2 have central organizational and coordinating roles. KREPA1 interacts with KREL2 and KRET2, and subcomplexes comprised primarily of these three proteins accurately catalyze the U addition and RNA ligation steps of insertion editing *in vitro* [18,26]. Similarly, KREPA2 interacts with KREL1 and KREPC2 (a possible ExoUase [18]), and subcomplexes comprised primarily of these three proteins accurately catalyze the U removal and RNA ligation steps of deletion editing *in vitro* [1,4,18,54]. The *Leishmania* ortholog of KREPC2 lacks the C-terminal EEP domain. Knock-ins of KREPA2 with mutated zinc-finger 1 or zinc-finger 2 domains in *L. tarentolae* result in substantial or partial disruption of the editosome, respectively, indicating that the zinc-finger domains are involved in protein–protein interaction [56]. KREPA1 and KREPA2 provide structural linkages for four enzymes and enhance their activities, perhaps by providing OB-fold domains (gray) that increase substrate specificity and catalysis [18,26]. Together with the finding that the U addition, U removal, and ligation steps together contribute to the accuracy of RNA editing [12,40], these observations have led to the hypothesis that the OB-fold domains of KREPA1 and KREPA2 provide flexible 'toggles' that undergo conformational changes that sequentially expose substrate-binding and active sites (red squares) for the ordered U addition or deletion and RNA ligation steps of editing [18]. KREPB5 is essential for the integrity of the complex [20] and is therefore shown in between the two subcomplexes, although no direct binding partners have been identified. Note that the two-domain structures of the proteins are hypothetical.

KREL2 and KREL1 from the editosome but to their disappearance altogether [47,48,54]. This implies that these proteins are degraded if they are not integrated into 20S editosomes.

The 20S editosome proteins differ in their importance for editosome integrity. Complexes of 20S remain after loss of KREL1, KREL2 or KRET2 [29,48,55]; however, knockdown of KREPA1 results in editosomes of ~15S [48], inactivation of KREPA2 substantially disrupts the 20S editosome [54,56], and loss of KREPB5 results in complete loss of the 20S editosome [20]. By contrast, pre-mRNA and gRNA are not required for 20S editosomes that catalyze *in vitro* editing [57].

The organization of endonucleases in the editosome is unknown and the loss of this activity on knockdown of KREPA2 expression might not be surprising given the substantial disruption of the 20S editosome [54]. Many components of the editosome are not yet localized in the structural map (Figures 2, 4), and the stoichiometry of the complex components is unknown. Indeed, it is likely that the 20S editosome has dynamic and alternative organizations and perhaps differs in composition during editing and the life cycle.

Other proteins and complexes

KRET1 is present in complexes that catalyze addition of the 3' oligo(U) tail to gRNAs. In *L. tarentolae*, most KRET1 is present in a complex of ~500 kDa (~10S) containing three or four KRET1 molecules, and a small amount is present in a complex of ~700 kDa of unknown composition that can be isolated by biochemical methods [27]. Recombinant KRET1 forms active oligomers *in vitro*, and C-terminally deleted KRET1 variants form dimeric complexes that are initially active but unstable. This behavior is unlike that of other members of the DNA polymerase-β superfamily, which function as monomers [27,28]. KRET1 complexes can associate with 20S editosomes via an RNase-sensitive link, which has led to the suggestion that this protein might function not only in the addition of gRNA oligo(U) tails but also in the transport of gRNAs into the 20S editosome [27].

Two related RNA-binding proteins, MRP1 and MRP2 (previously called gBP21 and gBP25), which were identified initially by gRNA crosslinking and subsequently in several kinetoplastids by database analysis, can anneal complementary RNAs and have roles that affect the abundance of edited RNA [58–61]. These proteins co-immunoprecipitate and form stable heterotetramers that promote RNA annealing [59,60]. TAP-tagged MRP1 is present in complexes containing small amounts of RNA editing ligases (an association, like the KRET1 complex interaction above, that is abolished by RNase treatment) and substoichiometric amounts of three proteins of 55–60 kDa, termed AP1, AP2 and AP3, [7,60]. MRP1 binds RNAs nonspecifically [60], but catalyzes a matchmaking type of complementary RNA annealing *in vitro*, and thus has been suggested to facilitate base-pairing between gRNAs and their cognate pre-mRNAs [62]. Accordingly, immunoprecipitates of MRP1 and MRP2 contain gRNAs [60,63]; in addition, monoclonal antibodies specific for MRP1 immunoprecipitate *in vitro* editing activity that is abolished by nuclease treatment [63] and inhibit editing activity *in vitro* [64].

MRP1-null mutants of the bloodstream form of *T. brucei* are viable and have slightly reduced levels of edited RNA but cannot progress to the insect form of the organism [64]. RNAi knockdown of MRP2 expression, either alone or in combination with knockdown of MRP1, inhibits cell growth, differentially affects the abundance of edited RNAs, and affects the abundance of RNAs that do not undergo editing [61]. This pattern resembles that seen after knockdown of the RNA-binding protein RBP16 (see below), although the level of this protein is unaffected in the MRP1 and MRP2 knockdowns. Taken together, these results imply that MRP1 and MRP2 have various roles, including the use of gRNA via a matchmaking activity that might help to regulate editing (see below), RNA turnover, and perhaps polycistronic pre-mRNA processing.

Three additional mitochondrial proteins, RBP16, RNA editing-associated protein-1 (REAP-1) and TbRGG1, might have roles that affect edited RNAs. All three proteins bind RNA but do not seem to be stably associated with the 20S editosome, KRET1 complex or MRP complex, although they might function by transient association with them. RBP16 has an affinity for oligo(U), contains a cold-shock domain

that is present in bacterial proteins that resolve RNA secondary structures, and can bind gRNAs, rRNAs and mRNAs [65,66]. RNAi knockdown of RBP16 in the insect form of *T. brucei* results in an accumulation of pre-edited Cyb mRNA and a reduction of edited Cyb mRNA, in addition to a reduction of mitochondrial mRNAs that do not get edited; however, gRNA levels are not affected by RBP16 knockdown [67]. This pattern closely resembles the effects of the knockdown of MRP1 and MRP2 expression, suggesting that RBP16 has a role in RNA turnover and perhaps in gRNA use, possibly in association with the MRPs.

REAP-1 is a protein of ~45 kDa that primarily is present in 35–40S complexes, generally co-fractionates with RNA ligase and TUTase activities, and preferentially binds to pre-edited RNAs rather than to RNAs, gRNAs or 'never-edited' RNAs [68,69]. Monoclonal antibodies specific for REAP-1 inhibit *in vitro* editing, implying that REAP-1 has a role in this process. REAP-1 has also been proposed to have a role in transporting pre-edited RNAs into the editing complex. TbRGG1 is a mitochondrial protein of ~75 kDa that contains five repeats of an Arg-Gly-Gly (RGG) motif that is conserved in some RNA-binding proteins [70]. Its co-sedimentation with *in vitro* deletion editing activity, and the finding that its RGG domain preferentially binds to oligo(U), has led to the suggestion that it might have a role in editing. No gene deletion or knockdown experiments for REAP-1 or TbRGG1 have been published at this time.

Most KREPA1, KREPA2, KREPA3 and KREL1 proteins are present in 20S editosomes, as seen in western blots probed with monoclonal antibodies [1], but some editing activities also peak at ~40S [71,72]. The relationship between the two peaks is unclear and the protein composition of the 40S peak is not well explored, but the two complexes might differ in their content of gRNA and edited and unedited mRNA. The 20S editosomes have been proposed to associate with gRNA and pre-mRNA and their associated proteins to form the 40S complex [71]. This association might involve the many complexes and proteins described above. In addition, polycistronic gRNA transcripts are processed into individual gRNAs in complexes that sediment at ~20S [73]; thus, these transcripts might be associated with 20S editosomes and/or the KRET1 or MRP complexes. However, gRNAs with oligo(U) tails sediment at ~40S [74] and might represent the associations among these complexes, perhaps via RNA as suggested [7].

Editing is regulated during the life cycle not by controlling gRNA abundance but more probably by controlling gRNA use [75]. The regulatory mechanism is unknown, but it probably involves the complexes and proteins described above. Editing is likely to be integrated with other mitochondrial RNA processing steps, such as the maturation of polycistronic pre-mRNAs, which can be edited before cleavage [76]; RNA turnover (e.g. see the effect of RNAi knockdown of MRP1, MRP2, and RBP16 described above); and possibly the maturation of rRNAs, which have added 3′ oligo(U) tails [77]. Such integration might explain why numerous nucleases are present in the 20S editosome.

Concluding remarks and perspectives

The organization of the editosome seems (i) to enhance the efficiency of the editing reactions, which is advantageous given the many hundreds of sites that get edited; (ii) to provide the basis for discriminating between insertion and deletion editing sites, which are intermixed in blocks of sequence specified by single gRNAs; (iii) to avoid opposing catalytic activities, such as cleavage or ligation, or the removal or addition of U; and (iv) to ensure that the steps of editing of a site occur in the correct order. The editosome might also proofread editing at single sites and in blocks of sequence specified by single gRNAs, thereby ensuring accurate editing. In addition, confinement of the catalytic activities within such a multiprotein complex not only facilitates the individual steps but can also confine activities, such as nucleolysis or RNA ligation, that might be detrimental if free in the cell.

The U insertion or deletion type of RNA editing is restricted to and characteristic of trypanosomatids. Several of the closest known homologs of editosome proteins function in DNA repair, which is superficially similar to editing in terms of its orderly cleavage, nucleotide excision, nucleotide addition and ligation, and which is catalyzed by a multiprotein complex that contains a coordinating protein [78]. This similarity implies a common ancestry and, together with the resemblance of editosome components to both bacterial and eukaryotic proteins, the development of editing from other processes. The similarity between the editing and T4 phage RNA ligases suggests that horizontal transfer might have contributed to the development of editing. Regardless of its origin, editing seems to have provided a selective advantage to trypanosomatids. It is essential because every situation in which editing is inactivated has proved to be lethal, implying that editing might be a useful drug target. Indeed, drugs that are effective against trypanosomatids localize in the mitochondrion and thus might target some aspect of RNA editing or its consequences.

References

1 Panigrahi, A.K. *et al*. (2001) Four related proteins of the *T. brucei* RNA editing complex. *Mol. Cell. Biol.* 21, 6833–6840
2 Panigrahi, A.K. *et al*. (2001) Association of two novel proteins, *Tb*MP52 and *Tb*MP48, with the *Trypanosoma brucei* RNA editing complex. *Mol. Cell. Biol.* 21, 380–389
3 Panigrahi, A.K. *et al*. (2003) Identification of novel components of *Trypanosoma brucei* editosomes. *RNA* 9, 484–492
4 Aphasizhev, R. *et al*. (2003) Isolation of a U-insertion/deletion editing complex from *Leishmania tarentolae* mitochondria. *EMBO J.* 22, 913–924
5 Rusche, L.N. *et al*. (1997) Purification of a functional enzymatic editing complex from *Trypanosoma brucei* mitochondria. *EMBO J.* 16, 4069–4681
6 Rusché, L.N. *et al*. (2001) The two RNA ligases of the *Trypanosoma brucei* RNA editing complex: cloning the essential Band IV gene and identifying the Band V gene. *Mol. Cell. Biol.* 21, 979–989
7 Simpson, L. *et al*. (2004) Mitochondrial proteins and complexes in *Leishmania* and *Trypanosoma* involved in U-insertion/deletion RNA editing. *RNA* 10, 159–170

8 Worthey, E.A. *et al*. (2003) Comparative analysis of editosome proteins in trypanosomatids. *Nucleic Acids Res.* 31, 6392–6408

9 Seiwert, S.D. *et al*. (1996) Direct visualization of uridylate deletion *in vitro* suggests a mechanism for kinetoplastid RNA editing. *Cell* 84, 831–841

10 Kable, M.L. *et al*. (1996) RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA. *Science* 273, 1189–1195

11 Piller, K.J. *et al*. (1997) Resolution of the RNA editing gRNA-directed endonuclease from two other endonucleases of *Trypanosoma brucei* mitochondria. *RNA* 3, 279–290

12 Lawson, S. *et al*. (2001) The specificity of nucleotide removal during RNA editing in *Trypanosoma brucei*. *RNA* 7, 1793–1802

13 Leung, S.S. and Koslowsky, D.J. (2001) Interactions of mRNAs and gRNAs involved in trypanosome mitochondrial RNA editing: structure probing of an mRNA bound to its cognate gRNA. *RNA* 7, 1803–1816

14 Salavati, R. *et al*. (2002) Endoribonuclease activities of *Trypanosoma brucei* mitochondria. *Mol. Biochem. Parasitol.* 120, 23–31

15 Salavati, R. *et al*. (2001) Mitochondrial ribonuclease P activity of *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 115, 109–117

16 Alfonzo, J.D. *et al*. (1998) Purification and characterization of MAR1. A mitochondrial associated ribonuclease from *Leishmania tarentolae*. *J. Biol. Chem.* 273, 30003–30011

17 Cruz-Reyes, J. *et al*. (1998) *T. brucei* RNA editing: adenosine nucleotides inversely affect U-deletion and U-insertion reactions at mRNA cleavage. *Mol. Cell* 1, 401–409

18 Schnaufer, A. *et al*. (2003) Separate insertion and deletion sub-complexes of the *Trypanosoma brucei* RNA editing complex. *Mol. Cell* 12, 307–319

19 Nicholson, A.W. (1999) Function, mechanism and regulation of bacterial ribonucleases. *FEMS Microbiol. Rev.* 23, 371–390

20 Wang, B. *et al*. (2003) TbMP44 is Essential for RNA editing and structural integrity of the editosome in *Trypanosoma brucei*. *Eukaryot. Cell* 2, 578–587

21 Igo, R.P., Jr. *et al*. (2002) Role of uridylate-specific exoribonuclease activity in kinetoplastid RNA editing. *Eukaryot. Cell* 1, 112–118

22 Cruz-Reyes, J. *et al*. (2001) Trypanosome RNA editing: simple guide RNA features enhance U deletion 100-fold. *Mol. Cell. Biol.* 21, 884–892

23 Aphasizhev, R. and Simpson, L. (2001) Isolation and characterization of a U-specific $3'$–$5'$ exonuclease from mitochondria of *Leishmania tarentolae*. *J. Biol. Chem.* 276, 21280–21284

24 Burgess, M.L.K. and Stuart, K. (2000) Sequence bias in edited kinetoplastid RNAs. *RNA* 6, 1492–1497

25 Igo, R.P., Jr. *et al*. (2002) RNA sequence and base pairing effects on insertion editing in *Trypanosoma brucei*. *Mol. Cell. Biol.* 22, 1567–1576

26 Ernst, N.L. *et al*. (2003) TbMP57 is a $3'$ terminal uridylyl transferase (TUTase) of the *Trypanosoma brucei* editosome. *Mol. Cell* 11, 1525–1536

27 Aphasizhev, R. *et al*. (2002) Trypanosome mitochondrial $3'$ terminal uridylyl transferase (TUTase): the key enzyme in U-insertion/deletion RNA editing. *Cell* 108, 637–648

28 Aphasizheva, I. *et al*. (2004) RNA-editing terminal uridylyl transferase 1: identification of functional domains by mutational analysis. *J. Biol. Chem.* 279, 24123–24130

29 Aphasizhev, R. *et al*. (2003) A tale of two TUTases. *Proc. Natl. Acad. Sci. U. S. A.* 100, 10617–10622

30 Burgess, M.L. *et al*. (1999) Kinetoplastid RNA editing does not require the terminal $3'$ hydroxyl of guide RNA, but modifications to the guide RNA terminus can inhibit *in vitro* U insertion. *RNA* 5, 883–892

31 Shuman, S. and Schwer, B. (1995) RNA capping enzyme and DNA ligase: a superfamily of covalent nucleotidyl transferases. *Mol. Microbiol.* 17, 405–410

32 McManus, M.T. *et al*. (2001) Identification of candidate mitochondrial RNA editing ligases from *Trypanosoma brucei*. *RNA* 7, 167–175

33 Schnaufer, A. *et al*. (2001) An RNA ligase essential for RNA editing and survival of the bloodstream form of *Trypanosoma brucei*. *Science* 291, 2159–2162

34 Ho, C.K. *et al*. (2004) Structure and mechanism of RNA ligase. *Structure (Camb.)* 12, 327–339

35 Sabatini, R. and Hajduk, S.L. (1995) RNA ligase and its involvement in guide RNA/mRNA chimera formation. Evidence for a cleavage–ligation mechanism of *Trypanosoma brucei* mRNA editing. *J. Biol. Chem.* 270, 7233–7240

36 Seiwert, S.D. and Stuart, K. (1994) RNA editing: transfer of genetic information from gRNA to precursor mRNA *in vitro*. *Science* 266, 114–117

37 Cruz-Reyes, J. and Sollner-Webb, B. (1996) Trypanosome U-deletional RNA editing involves guide RNA-directed endonuclease cleavage, terminal U exonuclease, and RNA ligase activities. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8901–8906

38 Cruz-Reyes, J. *et al*. (2002) Distinct functions of two RNA ligases in active *Trypanosoma brucei* RNA editing complexes. *Mol. Cell. Biol.* 22, 4652–4660

39 Blanc, V. *et al*. (1999) The mitochondrial RNA ligase from *Leishmania tarentolae* can join RNA molecules bridged by a complementary RNA. *J. Biol. Chem.* 274, 24289–24296

40 Igo, R.P., Jr. *et al*. (2000) Uridylate addition and RNA ligation contribute to the specificity of kinetoplastid insertion RNA editing. *Mol. Cell. Biol.* 20, 8447–8457

41 Lake, J.A. *et al*. (1988) Evolution of parasitism: kinetoplastid protozoan history reconstructed from mitochondrial rRNA gene sequences. *Proc. Natl. Acad. Sci. U. S. A.* 85, 4779–4783

42 Doherty, A.J. and Suh, S.W. (2000) Structural and mechanistic conservation in DNA ligases. *Nucleic Acids Res.* 28, 4051–4058

43 Suck, D. (1997) Common fold, common function, common origin? *Nat. Struct. Biol.* 4, 161–165

44 Odell, M. *et al*. (2000) Crystal structure of eukaryotic DNA ligase-adenylate illuminates the mechanism of nick sensing and strand joining. *Mol. Cell* 6, 1183–1193

45 Gao, G. and Simpson, L. (2003) 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.* 278, 27570–27574

46 Huang, C.E. *et al*. (2001) Roles for ligases in the RNA editing complex of *Trypanosoma brucei*: band IV is needed for U-deletion and RNA repair. *EMBO J.* 20, 4694–4703

47 Drozdz, M. *et al*. (2002) TbMP81 is required for RNA editing in *Trypanosoma brucei*. *EMBO J.* 21, 1791–1799

48 O'Hearn, S. *et al*. (2003) *Trypanosoma brucei* RNA editing complex: band II is structurally critical and maintains band V ligase, which is nonessential. *Mol. Cell. Biol.* 23, 7909–7919

49 Palazzo, S.S. *et al*. (2003) Kinetoplastid RNA editing ligases: complex association, characterization, and substrate requirements. *Mol. Biochem. Parasitol.* 127, 161–167

50 Koslowsky, D.J. *et al*. (1991) Cycles of progressive realignment of gRNA with mRNA in RNA editing. *Cell* 67, 537–546

51 Deng, J. *et al*. (2004) High resolution crystal structure of a key editosome enzyme from *Trypanosoma brucei*: RNA editing ligase 1. *J. Mol. Biol.* 343, 601–613

52 Missel, A. *et al*. (1997) Disruption of a gene encoding a novel mitochondrial DEAD-box protein in *Trypanosoma brucei* affects edited mRNAs. *Mol. Cell. Biol.* 17, 4895–4903

53 Panigrahi, A.K. *et al*. (2003) Mass spectrometric analysis of the editosome and other multiprotein complexes in *Trypanosoma brucei*. *J. Am. Soc. Mass Spectrom.* 14, 728–735

54 Huang, C.E. *et al*. (2002) Assembly and function of the RNA editing complex in *Trypanosoma brucei* requires band III protein. *Mol. Cell. Biol.* 22, 3194–3203

55 Stuart, K.D. *et al*. (2002) Composition of the editing complex of *Trypanosoma brucei*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 357, 71–79

56 Kang, X. *et al*. (2004) 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.* 279, 3893–3899

57 Domingo, G.J. *et al*. (2003) Dyskinetoplastic *Trypanosoma brucei* contain functional editing complexes. *Eukaryot. Cell* 2, 569–577

58 Koller, J. *et al*. (1997) *Trypanosoma brucei* gBP21: an arginine-rich mitochondrial protein that binds to guide RNA with high affinity. *J. Biol. Chem.* 272, 3749–3757

59 Blom, D. *et al*. (2001) 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.* 29, 2950–2962

60 Aphasizhev, R. *et al*. (2003) 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* 9, 62–76

61 Vondruskova, E. *et al.* RNA interference analyses suggest a transcript-specific regulatory role for MRP1 and MRP2 in RNA editing and other RNA processing in *Trypanosoma brucei*. *J. Biol. Chem.* (in press)

62 Müller, U.F. *et al.* (2001) Annealing of RNA editing substrates facilitated by guide RNA-binding protein gBP21. *EMBO J.* 20, 1394–1404

63 Allen, T.E. *et al.* (1998) Association of guide RNA binding protein gBP21 with active RNA editing complexes in *Trypanosoma brucei*. *Mol. Cell. Biol.* 18, 6014–6022

64 Lambert, L. *et al.* (1999) The involvement of gRNA-binding protein gBP21 in RNA editing – an *in vitro* and *in vivo* analysis. *Nucleic Acids Res.* 27, 1429–1436

65 Hayman, M.L. and Read, L.K. (1999) *Trypanosoma brucei* RBP16 is a mitochondrial Y-box family protein with guide RNA binding activity. *J. Biol. Chem.* 274, 12067–12074

66 Pelletier, M. *et al.* (2000) RNA-binding properties of the mitochondrial Y-box protein RBP16. *Nucleic Acids Res.* 28, 1266–1275

67 Pelletier, M. and Read, L.K. (2003) RBP16 is a multifunctional gene regulatory protein involved in editing and stabilization of specific mitochondrial mRNAs in *Trypanosoma brucei*. *RNA* 9, 457–468

68 Madison-Antenucci, S. *et al.* (1998) Kinetoplastid RNA-editing-associated protein 1 (REAP-1): a novel editing complex protein with repetitive domains. *EMBO J.* 17, 6368–6376

69 Madison-Antenucci, S. *et al.* (2002) Editing machines: the complexities of trypanosome RNA editing. *Cell* 108, 435–438

70 Vanhamme, L. *et al.* (1998) *Trypanosoma brucei* TBRGG1, a mitochondrial oligo(u)-binding protein that co-localizes with an *in vitro* RNA editing activity. *J. Biol. Chem.* 273, 21825–21833

71 Pollard, V.W. *et al.* (1992) Native mRNA editing complexes from *Trypanosoma brucei* mitochondria. *EMBO J.* 11, 4429–4438

72 Corell, R.A. *et al.* (1996) Complexes from *Trypanosoma brucei* that exhibit deletion editing and other editing-associated properties. *Mol. Cell. Biol.* 16, 1410–1418

73 Grams, J. *et al.* (2000) Processing of polycistronic guide RNAs in associated with RNA editing complexes in *Trypanosoma brucei*. *EMBO J.* 19, 5525–5532

74 McManus, M.T. *et al.* (2000) *Trypanosoma brucei* guide RNA poly(U) tail formation is stabilized by cognate mRNA. *Mol. Cell. Biol.* 20, 883–891

75 Stuart, K. *et al.* (1997) RNA Editing in kinetoplastid protozoa. *Microbiol. Mol. Biol. Rev.* 61, 105–120

76 Koslowsky, D.J. and Yahampath, G. (1997) Mitochondrial mRNA 3′ cleavage/polyadenylation and RNA editing in *Trypanosoma brucei* are independent events. *Mol. Biochem. Parasitol.* 90, 81–94

77 Adler, B.K. *et al.* (1991) Modification of *Trypanosoma brucei* mitochondrial rRNA by posttranscriptional 3′ polyuridine tail formation. *Mol. Cell. Biol.* 11, 5878–5884

78 Marsin, S. *et al.* (2003) Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1. *J. Biol. Chem.* 278, 44068–44074

79 Cherepanov, A.V. and de Vries, S. (2002) Kinetic mechanism of the Mg^{2+}-dependent nucleotidyl transfer catalyzed by T4 DNA and RNA ligases. *J. Biol. Chem.* 277, 1695–1704