Curr Genet (2005) 48: 277–299
DOI 10.1007/s00294-005-0027-0

REVIEW ARTICLE

Julius Lukeš · Hassan Hashimi · Alena Zíková

Unexplained complexity of the mitochondrial genome and transcriptome in kinetoplastid flagellates

Received: 5 August 2005 / Revised: 3 September 2005 / Accepted: 7 September 2005 / Published online: 8 October 2005

Abstract Kinetoplastids are flagellated protozoans, whose members include the pathogens *Trypanosoma brucei*, *T. cruzi* and *Leishmania* species, that are considered among the earliest diverging eukaryotes with a mitochondrion. This organelle has become famous because of its many unusual properties, which are unique to the order Kinetoplastida, including an extensive kinetoplast DNA network and U-insertion/deletion type RNA editing of its mitochondrial transcripts. In the last decade, considerable progress has been made in elucidating the complex machinery of RNA editing. Moreover, our understanding of the structure and replication of kinetoplast DNA has also dramatically improved. Much less however, is known, about the developmental regulation of RNA editing, its integration with other RNA maturation processes, stability of mitochondrial mRNAs, or evolution of the editing process itself. Yet the profusion of genomic data recently made available by sequencing consortia, in combination with methods of reverse genetics, hold promise in understanding the complexity of this exciting organelle, knowledge of which may enable us to fight these often medically important protozoans.

Keywords Kinetoplast · *Trypanosoma* · RNA editing · Mitochondrion

The peculiarities of the kinetoplastid mitochondrion

The mitochondrion of kinetoplastid protozoa is truly remarkable. Considering the knowledge that accumulated so far about its structure and functions, it qualifies as one of the most studied organelles. With the aim to summarize the progress in the field during the last decade, a substantial part of this review addresses functions of proteins involved in the maintenance and replication of mitochondrial (mt) DNA, termed kinetoplast (k) DNA in kinetoplastid protozoa, as well as in transcription, editing and processing of kinetoplast (k) RNA.

From all organisms studied so far, the jakobid protist *Reclinomonas americana* contains the highest number of protein-coding genes in its mt genome (Lang et al. 1997). Yet it is often overlooked that some kinetoplastid flagellates retain the largest amount of mtDNA per cell (Lukeš et al. 1998). Kinetoplastid flagellates belong to the phylum Euglenozoa, currently considered to be related to heteroloboseid amoeboflagellates and jakobids (Simpson and Roger 2004). It is comprised of three distinct clades: Kinetoplastida, Diplonemida and Euglenida, mutual relationships of which have yet to be resolved (Simpson et al. 2002). The order Kinetoplastida is the most intensively studied clade by far, containing important parasites causing diseases of humans, vertebrates and plants, as well as free-living species. It is subdivided into the monophyletic crown suborder Trypanosomatina and the early-branching suborder Bodonina (Doležel et al. 2000; Simpson et al. 2002). The latter suborder is now considered a paraphyletic assembly. Taxonomy proposed by Moreira et al. (2004), splitting bodonids into three clades, better reflects the relationships between these flagellates. As detailed below, we know a lot about the structure and function of kDNA and kRNA of parasitic trypanosomatids, but we know almost nothing about the giant kinetoplast genome of bodonids, the group which predominantly contains the non-parasitic evolutionary predecessors of trypanosomatids.

Euglenids are free-living, ecologically significant and omnipresent flagellates that usually contain a green plastid, whereas diplonemids are an often neglected,

Communicated by R. Bock

J. Lukeš (✉) · H. Hashimi · A. Zíková
Institute of Parasitology, Czech Academy of Sciences,
Faculty of Biology, University of South Bohemia,
Branišovská 31, 37005 České Budějovice, Czech Republic
E-mail: jula@paru.cas.cz
Tel.: +420-38-7775416
Fax: +420-38-5310388

species-poor group of free-living, commensalic and parasitic protists (Vickerman 2000). The uniqueness of kRNA editing in kinetoplastids (see the section on RNA editing below) is no exception among these ancient eukaryotes, as related diplonemids use another mechanism unprecedented for organelles—extensive *trans*-splicing of mtRNA (Marande et al. 2005). Curiously enough, the only firmly established fact about the mt genome of euglenids is that it can be recovered as a highly heterogeneous collection of small DNA molecules that may be independently transcribed (Gray et al. 2004). Some euglenozoan flagellates, such as *Cryptobia helicis*, *Bodo saltans*, *Trypanoplasma borreli* and *Diplonema papillatum* contain an extremely high amount of DNA in their single mitochondrion, which stains by DNA-specific dyes more intensely than the nuclear DNA (Lukeš et al. 2002; Marande et al. 2005). The available data therefore testify to an extreme diversity of mt genome structure and transcription within Euglenozoa, perhaps surpassing that of all other eukaryotes combined. It was suggested that kDNA content rose in the course of kinetoplastid evolution, as the organelle was invaded by a plasmid that eventually became indispensable (Lukeš et al. 1998). This hypothesis is against the dogma that postulates a gradual loss of mtDNA to the nucleus (Palmer 1997).

Because the spotlight has remained focused on kDNA replication and kRNA editing, less attention has been given to other key aspects of kinetoplastid mt biogenesis. In particular, little is known about oxidative phosphorylation and core metabolic pathways responsible for ATP production, with some long-standing paradigms being challenged by recent studies (for a recent review see also Besteiro et al. 2005). During a life cycle that involves a vertebrate host and a tse-tse fly vector, the mitochondrion of *Trypanosoma brucei*, the causative agent of African sleeping sickness, undergoes dramatic changes. While the bloodstream stage has a down-regulated organelle that has only a minor role in energy metabolism, it assumes a well-developped and fully active form in the insect (=procyclic) stage (for recent reviews see also Vickerman 1990; Schneider 2000).

Until recently, it was considered that pyruvate—the end product of glycolysis—can be further metabolized in the mitochondrion, but procyclics do not seem to use the Krebs cycle for oxidation of glucose-derived products (Van Weelden et al. 2003). Another postulate that procyclics produce ATP primarily by oxidative phosphorylation (Tielens and van Hellemond 1998) is not supported by new data (Besteiro et al. 2005). Although the proton gradient-generating cytochrome-containing respiratory chain is present along with an alternative oxidase, it does not seem to be the main source of ATP production. Instead, two substrate-level phosphorylation pathways have key roles in energy generation (Bochud-Allemann and Schneider 2002). Although several mitochondrial-encoded subunit genes of respiratory complex I are transcribed and edited (Estévez and Simpson 1999 and see below), very few nuclear-encoded subunits of this complex have so far been identified (A. Horváth et al., unpublished results). There are no functional data on complex I, and its mere participation in electron transport is a matter of controversy (Beattie and Howton 1996; Tielens and van Hellemond 1998; Hernandez and Turrens 1998). The fragmentary data on other complexes indicate a rather unorthodox subunit composition (Maslov et al. 2002; Horváth et al. 2005) and a stage-specific involvement of an alternative terminal oxidase (Chaudhuri et al. 1998).

Model kinetoplastids

Multiple different variations in kDNA structure, a defining character of the whole group, have been described, but small DNA circles are found in all kDNAs (with the single exception of *T. borreli*, which has large DNA circles of concatenated units). The circles are relaxed or supercoiled and are either free or are catenated into differently sized networks. Finally, they are either distributed throughout the organelle or confined to a single region (for recent review see also Lukeš et al. 2002). Recently, an extreme form of the kinetoplast that occupies significant portion of the cell has been described in *Perkinsiella* (Dyková et al. 2003).

Most of our knowledge about kDNA and kRNA comes from studies of the model trypanosomatids, *Crithidia fasciculata* and *Leishmania tarentolae*, that are non-pathogenic for humans, and the causative agents of African sleeping sickness—*T. brucei*. The first two model organisms are particularly useful for biochemical analyses that require cultivation in large amounts. Some trypanosomatids are rather refractory to genetic analysis (Robinson and Beverley 2003). Recently, however, this situation has dramatically changed, as methods of reverse genetics and RNA interference enable functional analysis of genes in *T. brucei*, but not in the other models (Ullu et al. 2004 and see below).

Among bodonids, only *B. saltans* and *T. borreli* are currently suitable for the studies of kDNA and kRNA, as the former can be cultivated in large volumes and the latter is the only bodonid available in axenic culture (Lukeš et al. 1994; Maslov and Simpson 1994; Gažiová and Lukeš 2003). As a consequence, our understanding of the kDNA replication and other mt processes, which appear to be extremely complicated and baroque, is a patchwork composed of the data derived from *T. brucei*, *L. tarentolae* and *C. fasciculata* (complemented to some extent by *T. cruzi*). We have included references to other trypanosomatids only in those cases in which substantial differences with the model organisms were described.

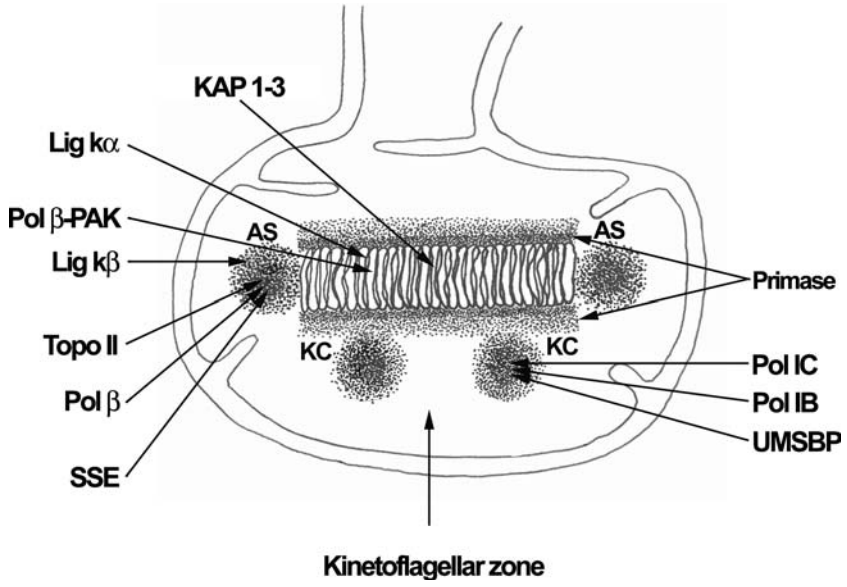

Fig. 1 Localization of kDNA-associated proteins. Kinetoplast associated proteins 1–3 (KAP1-3), involved in the packaging of kDNA, are localized throughout the kDNA disk. Proteins found so far in the antipodal sites (AS) are topoisomerase II (Topo II), structure-specific endonuclease 1 (SSE), DNA ligase kβ (Lig kβ), and DNA polymerases β (Polβ) and β-PAK (Polβ-PAK). The known constituents of the kinetoflagellar centres (KC) are DNA polymerases IB (Pol IB) and IC (Pol IC), and universal minicircle sequence binding protein (UMSBP). The uniquely localized DNA primase (Primase) is found above and below the kDNA disk, whereas DNA ligase kα (Lig kα) appears to be distributed throughout the kDNA disk

Kinetoplast DNA

kDNA structure

The kDNA of *C. fasciculata* is composed of about 25 large DNA circles termed maxicircles, and about 5,000 small circular molecules known as minicircles (Shapiro and Englund 1995) (Fig. 1). In trypanosomatid cells stained with any DNA-specific dye, kDNA is easily visible as a prominent disk-shaped spot located in the periflagellar region of the single elongated mitochondrion. As revealed by electron microscopy, it is a single giant network of mutually interlocked minicircles and maxicircles that has a highly ordered structure. The kDNA network is linked with the single flagellum by a filament system that traverses the mt membrane (Ogbadoyi et al. 2003; Liu et al. 2005). At least four histone-like proteins, called kinetoplast associated proteins 1–4 (KAP1-KAP4), have been implicated in stabilizing the kDNA network into a highly regular disk (Xu et al. 1996). Upon deletion of the KAP1 gene, the disk became less compact (Lukeš et al. 2001), whereas the concomitant loss of KAP2 and KAP3 had a general impact on the organelle and was lethal (Avliyakulov et al. 2004).

Maxicircles encode a set of subunits of mt respiratory complexes and two ribosomal RNA genes similar to the mt DNA of most eukaryotes (for review see Estévez and Simpson 1999), and their size varies from 20 kb in some trypanosomatids to 70 kb in *B. saltans* (Blom et al. 2000). All coding sequences are concentrated in a contiguous 17 kb stretch of the maxicircle sequence, called conservative region (Maslov et al. 1984), while the rest of the molecule, called the divergent region, is composed of repeated sequences of a variable structure and complexity (Sloof et al. 1992; Myler et al. 1993). Maxicircles appear to be mutually interlocked, forming a network that is intertwined with the minicircle network in a poorly understood manner (Shapiro 1993).

Also the size of the minicircles is species-specific, ranging from 0.5 to 10 kb (Shlomai 2004). Within a single kinetoplast, minicircles do not differ in size but differ in sequence and their only known function is to encode guide (g)RNAs indispensable for editing of mRNAs. Interestingly, some minicircles appear to have lost the gRNA genes and are still retained, perhaps because of a functional constraint of maintaining a certain genome size and topology of the network (Gao et al. 2001).

Comparative sequence analysis of minicircles revealed the presence of conserved sequence blocks (CSB1–3), which serve as replication origins (Ray 1989). Most, but not all, minicircles contain a bent helix region composed of regularly spaced adenine tracts (Marini et al. 1982) that distort the DNA and are specifically recognized by an endonuclease (Linial and Shlomai 1987). The function of this region is currently unknown. The catenated minicircles are stretched taut to the disk's axis, so that its thickness is determined by the size of minicircles, and its width by their number. The topology of minicircles has been described in considerable detail. In a stationary kinetoplast, every minicircle is catenated via a single interlock with three neighbours, creating a single network composed of thousands of mutually

concatenated DNA circles (Chen et al. 1995a, b). Such a structure remains manageable for the replication machinery since, in contrast to almost every circular DNA in nature, minicircles are not supercoiled and exist as covalently closed open circles (Rauch et al. 1993). Supercoils can be induced by the addition of intercalating dyes to isolated kDNA networks. To explain this extremely rare topological status of minicircles, an interesting theory was proposed that postulated that in order to get advantages associated with catenation of kDNA minicircles, the cell had to give up supercoiling (Rauch et al. 1993). As described below, segregation of minicircles during cell division is not very accurate, and catenation might have evolved to prevent the loss of individual minicircles due to unequal segregation. However, a comparative analysis of kDNA structures throughout the Kinetoplastida has shown that even in non-catenated kDNA, minicircles still exist as relaxed molecules (Lukeš et al. 2002).

kDNA replication

kDNA replication is probably the best understood case of replication of an organellar genome. The kDNA contains hundreds of minicircle sequence classes, each of them bearing a unique set of 1–4 gRNA genes that are essential for editing (see the chapter on RNA editing). Some rare gRNAs are encoded only by less than a dozen of minicircles out of the population of at least 5,000 (Maslov and Simpson 1992). Since incorrect segregation of a single essential minicircle would be lethal for one of the daughter cells, a sophisticated mechanism has developed for their book-keeping during replication.

Proteins involved in minicircle replication face the formidable task of replicating each minicircle only once and faithfully segregating the progeny into two daughter kDNA disks, all of which occurs within a giant network of thousands of mutually interlocked circular DNA molecules. As hundreds of proteins participate in this process, we have summarized just the general function of protein families shown to be involved. Most of the proteins have a distinct localization within or in the vicinity of the network, and may have overlapping functions with other members of the families (for recent reviews see also Klingbeil et al. 2001; Morris et al. 2001; Shlomai 2004; Liu et al. 2005).

During the nuclear S phase, individual minicircles are released in a vectorial manner from the network by the action of a still unidentified topoisomerase II, into a region between the kDNA disk and basal body of the flagellum, termed the kinetoflagellar zone (Fig. 1) (Drew and Englund 2001). Replication of the minicircle commences from a highly conserved CSB3 origin, from which the replication fork proceeds to form θ structure (Shapiro and Englund 1995; Klingbeil et al. 2001; Shlomai 2004). There is only a single gap in the continuously synthesized leading strand. As a consequence of discontinuous replication of the lagging strand, multiple gaps exist in the other half of the progeny. Replication of the continuous strand is primed by an RNA stretch produced by mt RNA polymerase, whereas replication of the complementary strand initiates from multiple RNA primers synthesized by DNA primase (Shlomai 2004). A number of additional proteins have been implicated in minicircle replication. Universal minicircle sequence binding protein (UMSBP) is an abundant protein that specifically binds the origin of minicircle replication (Abu-Elneel et al. 2001). UMSBP can bind DNA only as a monomer, but it is converted into an inactive dimer at a different redox state (Onn et al. 2004). Along with DNA primase and DNA and RNA polymerases, the UMSBP monomer assembles at the replication origins and initiates replication (Li and Englund 1997; Abu-Elneel et al. 2001; Klingbeil et al. 2002). The structure-specific endonuclease (SSE1) (Engel and Ray 1999), ribonuclease H (Ray and Hines 1995), DNA polymerase β (Torri et al. 1994) and DNA polymerase β-PAK (Saxowsky et al. 2003) have been associated with primer removal and filling of the gaps. DNA ligase β circularizes the linear replication intermediates (Sinha et al. 2004). Importantly, functional association of many of the above-mentioned enzymes has been confirmed by their co-localization with topoisomerase II. Although subtle differences in the positioning of proteins have been recently reported (Downey et al. 2005), they all localize within two opposite poles of the kDNA disk termed antipodal sites (Fig. 1).

As in all other genomes, replicative DNA synthesis is performed by DNA polymerase(s). However, while only a single mt DNA polymerase (pol γ) has been found in the organelle of other eukaryotes, trypanosomes harbor at least six different enzymes, belonging to two families. Although DNA polymerase IA may have redundant or overlapping functions, most of these polymerases appear to have specific roles in DNA replication and repair (Klingbeil et al. 2002). Such an array of different DNA polymerases is unprecedented and may reflect the challenges encountered by the protein machinery responsible for faithful replication of the kDNA network (Saxowsky et al. 2003). Similarly, there are multiple mt DNA ligases involved in replication and repair of gaps in minicircles (Klingbeil and Englund 2004), although not all of them appear to be essential. Yet different localization within the network of the two characterized ligases suggests that they have distinct functions (Downey et al. 2005). The down-regulation of DNA polymerases IB and IC, implicated in minicircle replication, is lethal (Klingbeil et al. 2002). Along with UMSBP, both enzymes have been localized to two protein centres situated below the kDNA disk in the kinetoflagellar zone that are distinct from the two antipodal sites. In order to distinguish these protein centres from the antipodal sites, we propose herein to name them "kinetoflagellar centers" (KC) (Fig. 1).

Orchestrated by a specialized topoisomerase II with the help of additional proteins (Wang and Englund 2001), the newly replicated minicircles return into the

network at the antipodal sites. It appears that several distinct topoisomerases may be involved in kDNA biogenesis, some of them having dual localization in the mitochondrion and nucleus (Gažiová and Lukeš 2002). So far, two distinct modes of reattachment have been documented. In *T. brucei*, the de novo synthesized minicircles continuously spread from the antipodal sites. However, in other trypanosomatids, such as *C. fasciculata*, the gapped minicircles first attach at the antipodal sites, subsequently appear around the disk's periphery and only gradually penetrate towards its centre (Guilbride and Englund 1998). While the former type of replication may involve a stationary type of kDNA disk, the latter is predicted to occur in a disk slowly rotating between the two antipodal sites, where replicated minicircles are being continuously reattached (Pérez-Morga and Englund 1993). Analyses of kDNA replication in several members of the genus *Trypanosoma* have revealed the types of replication intermediate between the two forms (stationary and rotating) described above (J. Lukeš et al., unpublished results). In addition, it was proposed that oscillation rather than continuous rotation may best explain the observed distribution patterns of newly replicated minicircles (Liu et al. 2005).

Little is known about the doubling of maxicircles, which appear to have a single replication origin and remain attached to the network throughout the process (Hajduk et al. 1984). Since the RNAi knock-down of RNA polymerase resulted in a decrease of maxicircles but not minicircles (Grams et al. 2002), this protein seems to be involved in the initiation of maxicircle replication. A narrow specificity to this substrate would mean that the enzyme has identical function in trypanosomes as in other eukaryotes that lack the equivalents of minicircles. As replication proceeds, the size of a fully replicated kDNA disk increases only slightly, whereas the valence of its minicircles doubles so that all of them become interlocked with six of their neighbours (Chen et al. 1995b). Each reattached minicircle retains at least one nick within the replication origin that remains unrepaired until all minicircles have been replicated (Birkenmeyer et al. 1987). Only then is this remaining gap synchronously filled in all minicircles, probably by DNA polymerase β-PAK (Saxowsky et al. 2003; Klingbeil and Englund 2004), and in a subsequent short period of time, sealed by a DNA ligase (Downey et al. 2005). This amazingly orchestrated action is followed by another enigmatic process, predictably involving an as yet unidentified topoisomerase II. The doubled kDNA network splits into two via a single straight scission, with the predicted topoisomerase II being arranged in a line along which the split occurs (Wang and Englund 2001).

Dyskinetoplastidy, transkinetoplastidy and exkinetoplastidy

At least two trypanosomes, *T. equiperdum* and *T. evansi*, exhibit a substantial loss of their mt genome, and can apparently survive without any kDNA. The bloodstream stages of both species are directly transmissible from one vertebrate host to another, with the insect stage being eliminated from their life cycle (for a recent review, see Schnaufer et al. 2001). The (total) absence of kDNA, known as dyskinetoplastidy, can also be induced by a number of drugs or by down-regulation of selected proteins in the kDNA containing-species such as *T. brucei* and *L. tarentolae* (Schnaufer et al. 2001; Wang and Englund 2001). The loss of kDNA causes the loss of mitochondrially encoded subunits of respiratory complexes that are indispensable for vital functions such as oxidative phosphorylation (Scheffler 1999). Therefore, the insect procyclic form cannot survive without its kDNA, whereas the dyskinetoplastic bloodform from mammals remains vital, since it relies on glycolysis for energy generation.

The phenomenon termed transkinetoplastidy is much less understood. It consists of dramatic changes in the population of maxicircles and minicircles induced by drugs such as sodium arsenite, tunicamycin and pentamidine (Lee et al. 1992; Basselin et al. 1998). A computer simulation demonstrated that random segregation of minicircles causes unexpectedly strong fluctuations of minicircle sequence classes in the time span of just thousands of generations (Simpson et al. 2000). However, drastic shifts in the population of minicircles during transkinetoplastidy, which requires only 15 generations (Lee et al. 1992), cannot be explained exclusively by random genetic drift. It is thus possible that the transkinetoplastic alterations in the kDNA represent a direct consequence of interference of some drugs with unknown mt functions.

Last but not least, an intriguing phenomenon associated with kDNA has been recently described in *T. cruzi*. Fragments of kDNA minicircles were found integrated within LINE-1 elements or β-globin genes of human patients suffering from chronic Chagas disease. Importantly, a similar transfer was observed in chickens and rabbits infected with *T. cruzi* (Nitz et al. 2004). No currently known cellular mechanism can mediate the transfer of minicircular DNA from the mitochondrion (with its double membrane) via the cytoplasm into the host cell nucleus, where it finally gets integrated into the nuclear genome. In line with the previously established nomenclature, we propose to name this emerging phenomenon "exkinetoplastidy". However, the implications of these results for the pathogenesis of Chagas disease will not be completely clear until the results of Nitz et al. (2004) are firmly corroborated.

Mitochondrial gene expression

In kinetoplasids, mt gene expression is the combined result of a complex set of processes that include polycistronic transcription, cleavage to release monocistrons followed and/or accompanied by U-insertion/deletion editing and polyadenylation of mRNAs and polyuridy-

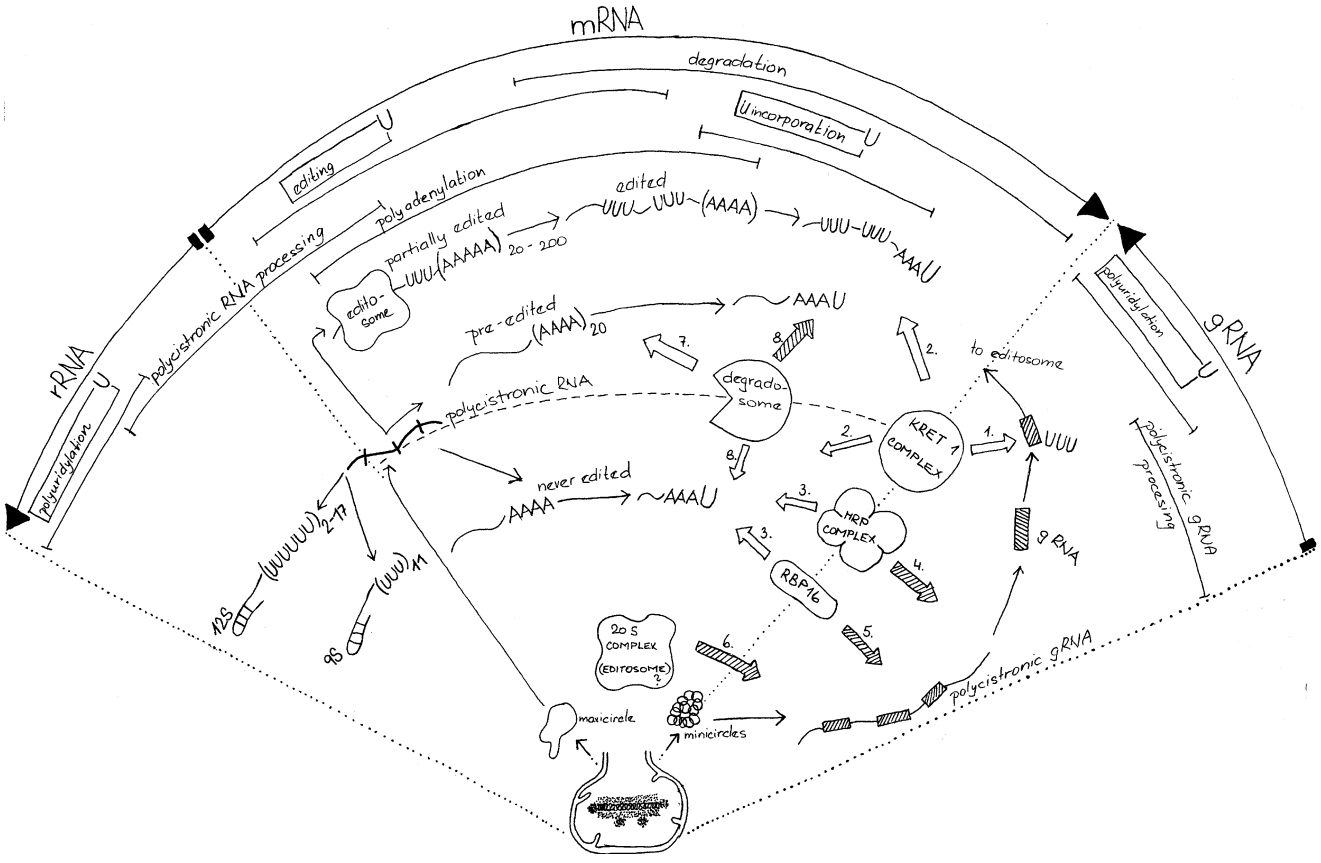

Fig. 2 Integration of kRNA processing events. This schematic diagram of the processing of RNA transcribed in the kinetoplast (*icon at the bottom*; see also Fig. 1) is divided into three sectors that correspond to the three major species of kRNA: rRNA, mRNA and gRNA. The mRNA sector is further subdivided into transcripts that are edited (*top*) or never-edited (*bottom*). Specific processing events are indicated at the periphery of the *semicircle*, with the *outermost elongated arrows* indicating in which order they occur. Many of these events are overlapping. Processes that involve UTP are *boxed*. Some proteins and complexes that play a role in kRNA processing are *depicted as icons*, with *thick numbered arrows* indicating their function with respect to the kRNA species in question. *Shaded arrows* are suggested functions that require further experimental evidence. The numbers correspond to the following events: (*1*) polyuridylation of gRNA; (*2*) Incorporation of UMP into poly(A) RNA; (*3*) stability of never-edited mRNA; (*4*) gRNA/mRNA matchmaking; (*5*) gRNA utilization; (*6*) polycistronic gRNA processing; (*7*) degradation of pre-edited mRNA with a poly(A)$_{20}$ tail; (*8*) degradation mRNAs with UMP incorporated into the poly(A) tail. The gRNAs are transcribed as polycistrons from minicircle kDNA. These transcripts are processed into individual molecules that are eventually polyuridylated by the KRET1 TUTase complex. Maxicircles transcribe polycistronic transcripts that are processed into mRNA and rRNA. The latter consist of 9S and 12S rRNAs that are eventually polyuridylated with U-tails of varying lengths. Edited mRNAs undergo U-insertion/deletion, which is facilitated by the editosome. Partially edited mRNAs with poly(A) tails of 20–200 nts are stabilized while pre-edited mRNAs with 20 nt poly(A) tails are degraded. UMP incorporation into edited and never-edited poly(A) tails enhances their degradation. The nature of this incorporation is not known, as is whether it occurs also in pre-edited transcripts

lation (poly[U]) of ribosomal RNAs (rRNAs) (Fig. 2). Given its overriding importance for the expression of mt genes, we will first focus on RNA editing.

RNA editing

Since the kinetoplast represents one of the first extranuclear DNA recorded in eukaryotes (Simpson 1973) it is ironic that complete characterization of the genes encoded in the mt genome lagged behind that in other eukaryotes, including humans (Anderson et al. 1981). Although potential orthologs of mt genes in other organisms were eventually found in maxicircle kDNA, many were aberrant, with detrimental frame-shifts or missing start codons, and some were not present at all (Benne et al. 1986). This latter situation even varied between trypanosomatid species, as three *L. tarentolae* maxicircle genes appeared to be absent in their corresponding locations in *T. brucei*, which were marked by stretches of sequence rich in guanosines (G) (Simpson et al. 1987).

How can these seemingly non-functional or even non-existing mt genes contribute to the biogenesis of the mitochondrion? The seminal work by Benne and coworkers (1986), who discovered that the transcript of cytochrome *c* oxidase subunit II (coxII) contained four uridine (U) residues not encoded in the gene, com-

menced the unravelling of this mystery. This modification was dubbed RNA editing since it repaired a -1 frameshift post-transcriptionally, restoring the appropriate open reading frame (ORF) by the insertion of U residues into predetermined parts of the message. A surge of reports following Benne's, focusing mainly on the trypanosomatids *T. brucei* and *L. tarentolae*, showed that 12 of the 20 kinetoplast genes produce transcripts that are (often extensively) edited (Estévez and Simpson 1999). Editing creates translatable ORFs in three possible ways:

(1) frameshift repairs, as originally discovered;
(2) generation of a start codon and/or several adjacent codons at the 5′ end of the mRNA by insertion of the appropriate U, as found in cytochrome *b* (cyB) (Feagin et al. 1987);
(3) creation of a whole ORF by the insertion of hundreds of Us and also the deletion of tens of Us. The third variation, referred to as pan-editing, is typified by the edited mRNA of coxIII, in which 547 Us are added and 41 Us removed (Feagin et al. 1988). Genes that encoded transcripts that are panedited were called cryptogenes, because long stretches of inherently intranslatable sequences are converted into ORFs by RNA editing. Depending on the species, a varying number of mRNAs is produced without any RNA editing at all. Such transcripts are referred to as 'never-edited'.

Thus, the mitochondrial genes that seemed to be missing or defective were actually not, but their mRNA had to be edited by U-insertion, and to a lesser extent U-deletion, in order to create recognizable ORFs. Although this process unveiled one mystery, it seemed to expose another: what supplies the information dictating where and how the edited mRNAs are modified, since U-insertion/deletion is not apparently determined by the cryptogene itself?

The mechanism of RNA editing

A collection of small RNAs found to be predominantly transcribed from the minicircles eventually solved the second mystery (Blum et al. 1990; Sturm and Simpson 1990a). These transcripts were dubbed guide (g) RNAs, because they provided the information for U-insertion and deletion at specific editing sites (ES) on the edited mRNA (Blum et al. 1990). The information for editing of specific transcripts stored within the primary structure of the gRNA, which is divided into three regions according to their function and content. The anchor region is a small stretch of about ten nucleotides in the 5′ third of the molecule, which 'anchor' the gRNA by hybridizing to a complementary sequence on the mRNA just downstream of the ES, and thus defines the specificity of the gRNA. The first mismatched nucleotide-pair marks an ES on the mRNA and the beginning of the information region on the gRNA, which provides the template for U-insertion/deletion. The transfer information from the guide to the message is based on both Watson–Crick and noncanonical G:U base pairing between the gRNA and mRNA (Blum et al. 1990; Sturm and Simpson 1990a). After completion of editing, the guiding region becomes complementary to the respective part of the edited mRNA, termed the "editing block". Finally, the 3′ part of the gRNA is comprised of the oligo(U) tail, which is added post-transcriptionally and is heterogeneous in length (Blum and Simpson 1990). This region is thought to anneal to the purine-rich pre-edited region, stabilizing the interaction between the gRNA and mRNA molecules.

Although the gRNAs provide the requisite information for U-insertion or deletion, they are not catalytic and thus proteins constitute the RNA editing machinery. An enzyme cascade orchestrates the individual reactions needed for this process (Blum and Simpson 1990). The initial step in RNA editing is the anchoring of a gRNA to its cognate mRNA just downstream of the first ES, forming a duplex. The ensuing endonucleolytic cleavage of the mRNA at the ES divides the transcript into 5′ and 3′ fragments. The gRNA acts to bridge the two via hybridization of the anchor domain with the 3′ fragment and interaction of the oligo(U) tail with the purine-rich pre-edited sequences in the 5′ fragment. What follows depends on whether U-insertion into or deletion from the mRNA is designated by the information domain in question.

Free UTP is the source of Us that is added to the 3′ hydroxyl group of the 5′ fragment in the case of U-insertion editing. The number of Us added is guided by the number of adenosines (As) and guanosines (Gs) just downstream of the gRNA anchor domain, since these residues base pair with the incoming nucleotide. This step ends when a U or cytosine (C) is encountered in the information domain. U-deletion occurs when Us are present on the 3′ end of the 5′ fragment that stick out of the gRNA:mRNA duplex. The bulging of the extra U(s) is further induced by the informational domain of the gRNA, which hybridizes to complementary nucleotides just 5′ of the Us. A 3′ → 5′ exonuclease prunes away the protruding U(s) up to the *bona fide* base pairs. Regardless of whether U-insertion or deletion is performed, the information inherent in the gRNA is transferred to the transcript at this point. The two mRNA fragments are rejoined with an RNA ligase, the final step in this cascade. The reactions described thus far only comprise a single round of editing. All pre-edited transcripts require several rounds since their ORFs are encrypted within multiple ESs. Furthermore, in pan-edited mRNAs the recruitment of multiple gRNAs is required to restore functional ORFs.

The polarity of ES selection along the edited block of a transcript is exceptional because it occurs in a 3′ to 5′ direction, in contrast to other molecular processes involving nucleic acids. During the initial characterization of edited mRNAs, the detection of partially edited intermediates provided snapshots of editing-in-progress

(Abraham et al. 1988; Sturm and Simpson 1990b). When clones of the intermediates of the same transcript were aligned, a pattern emerged in which the 3′ edited sequence gradually extended into the adjacent 5′ pre-edited part. The reason for this 3′ → 5′ polarity became apparent when sequence data of gRNAs of pan-edited transcripts showed that the anchoring domains of many gRNAs were not complementary to any part of the pre-edited mRNAs (Maslov and Simpson 1992; Corell et al. 1993). Instead, the target sequence for the subsequent gRNA anchor domain is created by U-insertions/deletions mediated by the previous gRNA, editing sequences just up-stream of the next ES on the transcript. Only when the mRNA is completely edited, it becomes ready for translation, since partially edited transcripts often lack essential 5′ sequences, such as the start codon (Maslov and Simpson 1992).

The information region of a given gRNA may specify 1 to 20 ESs. Whether editing of ESs within one region occurs 3′ → 5′ is still an open question. Many partially edited intermediates were isolated that did not exhibit strict 3′ → 5′ direction within these regions (Maslov and Simpson 1992; Sturm et al. 1992). Three explanations for this occurrence were offered by three different groups: (1) U-insertion/deletion is random and the correctly edited mRNA is chosen by perfect hybridization with the gRNA (Decker and Sollner-Webb 1990); (2) the order of U-insertion/deletion is dictated by local thermodynamic stabilities between base pairs in the gRNA:mRNA duplex, the intermediates being targeted for further editing since they inadequately hybridize the gRNA (Koslowsky et al. 1991); (3) editing does occur in the 3′ to 5′ direction, but misediting occasionally results from spurious gRNA binding or misguiding by the appropriate gRNA (Sturm et al. 1992).

Misedited mt transcripts are a logical side product of such a complex process. In addition to the latent imprecision of the essential G:U base pairing between the gRNA and mRNA, these molecules also have the potential to join together to form dead-end products termed chimeras (Blum et al. 1991). Chimeras result from the destabilization of the 3′ oligo(U) tail, which then comes into proximity with the 3′ fragment of the mRNA, to which it is eventually ligated (Seiwert et al. 1996; Burgess et al. 1999). Although detected in very low abundance among steady-state mRNAs (Riley et al. 1995), these aberrant products were initially believed to be intermediates in the alternative transesterification model of RNA editing (Blum et al. 1991; Cech 1991; Arts et al. 1995). This conceptually satisfying theory suggested that the oligo(U) tails of the gRNAs supplied the inserted Us in a reaction reminiscent of RNA splicing. A second model also called for the oligo(U) tail to supply the Us, although via endonucleolytic and RNA ligase activities (Blum et al. 1991). Although the presence of in vitro chimera-formation activity in mitochondrial lysates lent credence to these models (Blum et al. 1992; Koslowsky et al. 1992), more refined in vitro studies ultimately rejected them (see Stuart et al. 1997 for more on transesterification models).

In vitro RNA editing

The development of in vitro RNA editing systems, reproducing a full-round of editing, was a breakthrough in research on this topic, especially at a time when functional genomics in kinetoplastids did not exist. These systems allowed the elucidation of the basic molecular mechanisms underlying U-insertion/deletion and the validation of the enzyme cascade model. They also have become helpful tools in the identification and characterization of proteins involved in editing. The principle of these experiments will be discussed (for a more detailed account of this and related techniques, see Stuart et al. 2004).

The first functional in vitro system was established by Seiwert and Stuart (1994), which reproduced naturally occurring U-deletions within the pan-edited ATPase subunit 6 (A6) mRNA. It is composed of a substrate that contained part of the pre-edited A6 mRNA including an ES with four Us slated for removal, which was partnered with a synthetic gRNA directing this action with the wild-type sequence or variants thereof. Prior to the development of this assay, it had been established that mt lysates of kinetoplastids retain the enzymatic activities involved in RNA editing (Bakalara et al. 1989; Harris et al. 1992). After an improvement of this system, in which either the 5′ or 3′ end of the pre-mRNA is radiolabelled (Seiwert et al. 1996), the addition of these substrates to lysates obtained from *T. brucei* allowed a direct analysis of U-deletion editing.

This assay proved that base pairing between the gRNA and mRNA indeed directs the removal of the number of Us specified within the information region (Seiwert and Stuart 1994). Several products were observed during the course of the in vitro reactions, the features of which were consistent with the enzyme cascade model (Seiwert et al. 1996). When the 3′ end of the pre-mRNA was labelled, the 3′ cleavage product and religated edited mRNA were detected prior to the appearance of chimeras. When the 5′ fragment was labelled, the step-wise deletion of Us from the 5′ cleavage product could be visualized on a denaturing gel as a ladder of bands differing by one nucleotide. These results indicated that the editing catalytic activities were present in the mt lysates. Since chimeras appeared later than the edited A6 mRNA, they are an aberrant side-product instead of an editing intermediate. Furthermore, the processing of the Us at the end of the 5′ fragment pointed to the existence of a 3′ → 5′ U-exonuclease activity. An in vitro system for U-insertion editing soon followed, which was based on the addition of Us at an A6 ES just upstream of the site utilized in in vitro deletion (Kable et al. 1996). The principle is the same as that of the deletion assay, except that UTP was also added to the mix, showing that this free nucleotide

is the source of the inserted Us instead of the oligo(U) tail. It was deduced that enzymes with a terminal uridylyl transferase activity added the Us to the 5′ fragment. Chimeras were produced as well as an apparently unavoidable side product of in vitro editing.

Both in vitro assays were instrumental in understanding the mechanism of this process in vivo, although they are ultimately an approximation of what is actually going on in the organelle. The absence of multiple rounds of editing is one obvious aspect of in vitro editing that illustrates its limitations. The abundance of chimeras as side product represents another drawback, probably due to the relative inefficiency of the in vitro system considering they constitute a marginal portion of steady state kRNA (Riley et al. 1995). It should be mentioned that the formation of chimeras exploits the same enzymatic activities required for proper RNA editing (Rusché et al. 1995; Piller et al. 1997). The efficiency of in vitro editing was increased by developing a pre-cleaved pre-mRNA substrate, in which 5′ and 3′ pre-mRNA fragments annealed to a gRNA "bridge" were used to bypass the endonucleolytic cleavage step (Igo et al. 2000, 2002). This bridge RNA also differs from wild-type gRNA in that the 3′ oligo(U) tail is replaced with a sequence complementary to cognate 5′ fragment, which diminishes misediting that may occur from chimera formation and religation of unprocessed pre-mRNA (Igo et al. 2000). Although a further departure from in vivo editing, this modification has proven to be invaluable in characterizing the components of the 20S editosome, the multi-protein complex that encompasses the core enzymatic activities required for RNA editing. Since chimera formation is no longer the focus of editing research, recent in vitro editing studies typically use 5′-labelled 3′ pre-mRNA fragments, which does not detect these side-products.

The 20S editosome complex

The enzyme cascade model of RNA editing calls for the sequential action of certain enzymatic activities. The enzymes required for this process, listed in order of appearance, are: (1) an endonuclease that cleaves the mRNA at the ES, (2) a U-specific 3′ → 5′ exonuclease (exoUase) for deletion and a terminal uridylyl transferase (TUTase) for insertion editing and (3) an RNA ligase to rejoin the two fragments after processing. It was proposed that these proteins are assembled into a macromolecular complex, called the editosome, which would allow a more precise coordination of their activities (Stuart et al. 1989). Glycerol gradient fractionation of mt lysates showed that a number of these activities indeed co-sediment, a first indication of their association (Pollard et al. 1992; Peris et al. 1994). The existence of an editing complex was later confirmed with the aid of the in vitro deletion assay, which was used to probe the fractionated mt lysates of *T. brucei* and showed that the deletion activity peaks at 20S on glycerol gradients (Corell et al. 1996). This 20S *T. brucei* complex and its 25S counterpart in *L. tarentolae* (Peris et al. 1994) were the entry points into understanding the fundamental biochemistry of RNA editing. However, there is some debate as to whether this complex should be considered to be the 'editosome', or that there is more than one complex reflecting a varying composition of the in vivo editing machinery (Simpson et al. 2004). This idea will be revisited later in this review, and the complex containing the core editing activities will be referred to as the 20S editosome here, although it is also known as the L-complex (Aphasizhev et al. 2003c) to acknowledge that it probably may not represent the editosome as a whole. The current picture of the 20S complex, its components and structure, is described below (for recent reviews see also Simpson et al. 2004; Stuart et al. 2005).

Two RNA ligases were the first 20S editosome proteins to be directly visualized as 52 and 48 kDa proteins, respectively, taking advantage of their self-adenylation activity using [α-^{32}P] ATP (Sabatini and Hajduk 1995). As more data accumulated, it became apparent that other editosome proteins also occurred in pairs or sets (Stuart and Panigrahi 2002). These findings make sense in light of the strong evidence suggesting that the editosome is divided into two discrete subcomplexes devoted to either insertion or deletion editing (Schnaufer et al. 2003). This pattern shall become clear as the components of the 20S editosome are listed here. It is important to state that many of these proteins have several names, derived from the specific nomenclature of different laboratories and kinetoplastid species from which they were isolated. In this review, the nomenclature introduced in Stuart et al. (2005) shall be used, which also contains a Rosetta Stone for decoding the disparate terminology of the field (see Table 1).

Proteins with a gRNA-dependent endonuclease activity have proven to be the most difficult to identify in the purified 20S editosome. Several endonuclease activities have been detected in mt extracts from model trypanosomatids (Piller et al. 1997; Alfonzo et al. 1998; Salavati et al. 2001, 2002). ATP has an opposite effect on in vitro gRNA-directed cleavage depending on whether insertion or deletion editing substrates are used, and suggesting distinct endoribonucleases for each of these processes (Cruz-Reyes et al. 1998). Consistent with these reports, a number of isolated 20S editosome proteins have motifs that suggest a role as endonucleases: KREX1, KREX2 and KREPB1-5 (Panigrahi et al. 2003a; Aphasizhev et al. 2003b). The two KREX proteins have been recently renamed to reflect an established role as an exoUase (Kang et al. 2005), although a potential role as an endonuclease has not been investigated. These proteins will be further discussed. KREPB1-5 are a group of novel RNAse III proteins, containing a motif that may confer endonuclease activity, although KREPB4 and 5 have less similarity to this domain than the others. All five contain N-terminal U1-like zinc-finger domains, which may facilitate interaction with RNA or other proteins. They also have RNA

Table 1 A summary of the new nomenclature for Kinetoplastid RNA editing protein components of the 20S editosome, as introduced in Stuart et al. (2005)

The operational names used by different RNA editing groups are given alongside the new designations. Names in parenthesis are functional names that have also been used

New nomenclature	Stuart lab	Simpson lab	Sollner-Webb lab
KREPA1	TbMP81	LC-1	Band II
KREPA2	TbMP63	LC-4	Band III
KREPA3	TbMP42	LC-7b	Band VI
KREPA4	TbMP24	LC-10	
KREPA5	TbMP19		
KREPA6	TbMP18	LC-11	Band VII
KREPB1	TbMP90		
KREPB2	TbMP67		
KREPB3	TbMP61	LC-6a	
KREPB4	TbMP46	LC-5	
KREPB5	TbMP44	LC-8	
KREPB6	TbMP49	LC-7c	
KREPB7	TbMP47		
KREPB8	TbMP41		
KREX1 (REX1)	TbMP100	LC-2	
KREX2 (REX2)	TbMP99	LC-3	Band I
KREL1 (REL1)	TbMP52	LC-7a	Band IV
KREL2 (REL2)	TbMP48	LC-9	Band V
KRET1 (RET1)	3′TUTase		
KRET2 (RET2)	TbMP57	LC-6b	
KREH1	mHel61p		

binding domains adjacent to the RNAse III motif, KREPB1-3 containing a dsRNA binding domain whereas Pumilio-like regions that typically recognize specific mRNA sequences occur in KREPB4 and 5 (Worthey et al. 2003). RNAi knock-downs of KREPB5 did not result in a reduction of endonucleolytic activity, although it did result in the eventual destabilization of the 20S editosome (Wang et al. 2003). This result suggests that KREPB4 and 5, which have the less conserved RNAaseIII domains, are more likely structural proteins than endonucleases (Stuart et al. 2005). Although sequence data single out KREPB1-3 as potential 20S editosome endonucleases, experimental verification is required. Three other proteins that contain U1-like zinc fingers (KREPB6-8), do not possess RNAse III-like domains and are therefore presently excluded from the list of putative endonucleases (Worthey et al. 2003; Stuart et al. 2005).

Recombinant KREPA3 appears to have in vitro endo/exoribonuclease activities, with specificity for single-stranded U residues that loop out from flanking RNA double helices (Brecht et al. 2005). This finding is surprising for a protein whose only recognizable motif suggests a capacity to bind another protein and/or a single-stranded RNA, via zinc fingers and an oligonucleotide/oligosaccharide binding (OB)-fold domain (Worthey et al. 2003). Although in vivo and in vitro editing was substantially reduced in KREPA3 RNAi knock-down cells, the pre-cleaved in vitro editing assay was utilized, which does not directly test the gRNA-dependent endonuclease or exoUase activities.

The KREX1 and 2 proteins possess a 3′ → 5′ exonuclease domain, and have comparable molecular weights and high sequence identity (Worthey et al. 2005). Their role as the exoUase proteins is supported by the presence of KREX2 in the deletion subcomplex (Schnaufer et al. 2003) and RNAi down-regulation of KREX1 resulting in a significant reduction in in vitro deletion editing (Kang et al. 2005). In the latter report, in vitro deletion editing of pre-cleaved substrates was reconstituted using only recombinant versions of KREX1 and KREL1, one of the editosome RNA ligases. However, KREX1-silenced cells exhibited only slower growth, suggesting KREX2 can compensate for its absence. The presence of the endonuclease/exonuclease/phosphatase (EEP) domain in both KREPC proteins suggests that they may be multi-functional, although this domain is missing in *Leishmania* spp. KREX2 (Worthey et al. 2005; Kang et al. 2005). Perhaps *L. tarentolae* KREX1 serves as a substitute for the stunted KREX2 if this domain is necessary for deletion (Kang et al. 2005). The significance of two such similar proteins in the 20S editosome is still unknown.

There are two mitochondrial TUTases that are essential for RNA editing, KRET1 (Aphasizhev et al. 2002) and KRET2 (Ernst et al. 2003). Although both proteins are related, each having nucleotidyltransferase and poly(A) polymerase domains, in vitro assays (Ernst et al. 2003) and RNAi-mediated silencing of either transcript (Aphasizhev et al. 2003b) have shown that they have very different roles. KRET2 is the enzyme contributing TUTase activity to the 20S editosome and a component of the U-insertion subcomplex (Schnaufer et al. 2003). The protein catalyzes gRNA-directed addition of Us to the 3′ end of 5′ pre-cleaved editing substrates in vitro, preferentially to those with 3′ terminal purines (Ernst et al. 2003), which are typically at this position in pre-edited RNAs (Burgess and Stuart 2000). Interestingly, in the absence of a gRNA, KRET2 adds only a single U to these substrates (Ernst et al. 2003; Aphasizhev et al. 2003a), in sharp contrast to KRET1 which adds numerous Us, indicating that KRET1 adds Us in a gRNA-independent manner. Furthermore, RNAi ablation of KRET1 resulted in the

addition of shorter oligo(U) tails on gRNAs, ultimately inhibiting RNA editing (Aphasizhev et al. 2002, 2003b). KRET2 down-regulation also exhibits a decrease in editing without affecting the length of the oligo(U) tails. These results indicate that KRET1 is the TUTase responsible for gRNA oligo(U) tail formation. Additional data suggest that it is part of a complex that processes gRNAs (Simpson et al. 2003). KRET1 contains a zinc-finger domain that is not present in KRET2 (Ernst et al. 2003), which seems to be essential for enzymatic activity but not for interaction with other components of the (putative) complex (Aphasizheva et al. 2004).

The two 20S editosome RNA ligases, KREL1 and 2, are quite similar in sequence (McManus et al. 2000; Worthey et al. 2003). However, the localization of these enzymes hints at separate roles in RNA editing, as KREL1 is a part of the deletion subcomplex, while the insertion subcomplex contains KREL2 (Schnaufer et al. 2003). Biochemical evidence for a distinct role of KREL1 and KREL2 is that their activities exhibit different sensitivities to ATP and pyrophosphate, which correspond to the optimal conditions of in vitro deletion and insertion editing, respectively (Cruz-Reyes et al. 1998). Further experimental evidence suggests that KREL1 may have a role in RNA repair (Huang et al. 2001). KREL1 is essential for RNA editing in both procyclic (Rusché et al. 2001) and bloodstream forms (Schnaufer et al. 2001; Huang et al. 2001). Knock-out of the gene in the latter stage was lethal, a surprising discovery since the survival of dyskinetoplastic mutants implied that RNA editing was dispensable in this stage. On the other hand, KREL2 down-regulation did not significantly affect either RNA editing or cell viability (Drozdz et al. 2002; O'Hearn et al. 2003; Gao and Simpson 2003). This finding seemingly contradicted the theory that KREL1 and 2 exclusively participate in either deletion or insertion editing, respectively, when only silencing of the former is detrimental to the organism. Because edited mRNAs require both types of modification, one explanation would be that KREL2 does not function in RNA editing and that all editing functions in vivo are performed by KREL1 (Gao and Simpson 2003). Alternatively, both ligases are active in editing performed by the respective subcomplexes, but only KREL1 can compensate for the absence of KREL2, not vice versa (Schnaufer et al. 2003). As support for this hypothesis, KREL1 was shown to be active in both in vitro deletion and insertion assays, while KREL2 ligation was demonstrated only in the insertion assay (Schnaufer et al. 2003). The apparent flexibility of KREL1 in participating in both types of editing may be due to its demonstrated lack to of substrate specificity (Cruz-Reyes et al. 2002; Palazzo et al. 2003). A recently established system in which recombinant KRELs reconstitute in vitro editing in mitochondrial lysates depleted of these enzymes may be used to further investigate their function (Gao et al. 2005).

Both RNA ligases lack a C-terminal OB-fold, a domain that facilitates the binding of nucleic acids in DNA ligases (Schnaufer et al. 2003). The 20S editosome has a set of proteins that contain this motif, KREBA1-6 (Panigrahi et al. 2001a, b; Worthey et al. 2003). KREBA1-3 also contain N-terminal zinc fingers, while the other three are smaller and lack zinc fingers. Purification of the insertion and deletion subcomplexes by tandem-affinity purification (TAP) of proteins associated with each KREL, together with yeast-two hybrid and co-immunoprecipitation interaction studies, showed that KREBA1, 2 and 6 have key structural and functional roles (Schnaufer et al. 2003). The subcomplexes are linked to each other through the smallest of the three, KREPA6. The central component of the insertion subcomplex is KREPA1, serving as a connector between KREL2 and KRET2. RNAi-silencing of its transcript leads to loss of KREL2 and loss of in vitro insertion editing activity (Drozdz et al. 2002; O'Hearn et al. 2003). KREPA2 joins KREL1 and KREPC2, one of the putative exoUases, to form the deletion subcomplex. KREPA2-downregulation also leads to loss of the associated RNA ligase, affecting both in vitro deletion and insertion (Huang et al. 2002). The zinc fingers of KREPA2 were shown to facilitate protein–protein interaction, since over-expression of KREPA2-versions with mutations in either of these domains resulted in destabilization of the complex (Kang et al. 2004).

A mechanism for how the two subcomplexes within the 20S editosome facilitate RNA editing has been proposed based on the association of the RNA ligases within their respective subcomplexes (Schnaufer et al. 2003). The RNA ligases are proposed to be supplied in *trans* by their respective KREPA partners with the necessary OB-folds. This arrangement is a possible mechanism for the coordination of the U-insertion/deletion and ligation events: the OB domain of KREPA1 or 2 could serve to bind the exoUase or TUTase, together with the corresponding ligase to the gRNA:mRNA complex, bringing the RNA to be edited in close proximity of the appropriate activities.

The presence of an RNA helicase was proposed, since the unwinding of the gRNA:mRNA duplexes after editing is completed would be necessary for following steps such as editing of an adjacent ES or translation. This hypothesis was confirmed by the cloning of KREH1, a mitochondrial RNA helicase with the DEAD box motif (Missel et al. 1997). Procyclic knockouts for KREH1 demonstrated slower growth and a reduction in editing, which was rescued upon reintroduction of an ectopic copy of the gene. Since elimination of this helicase did not completely abolish RNA editing, it has been suggested that it is either non-essential for this process or that one or more helicases compensate for its loss. Database searches have found another putative helicase (Panigrahi et al. 2003b), providing some evidence in favour of the second possibility. Although KREH1 was found in 20S editosomes isolated by column chromatography and immunoprecipitation, it is not likely to be

a stable component of the complex, since it is absent from 20S preparations purified by the TAP-tagging procedures mentioned above (Panigrahi et al. 2003a).

Inconsistencies in the composition of purified 20S editosomes

Several laboratories undertook the isolation of the 20S editosome from *T. brucei* and identified constituent proteins, using different isolation and screening strategies (Rusché et al. 1997; Madison-Antenucci et al. 1998; Panigrahi et al. 2001a, b). These distinct approaches yielded 20S editosomes that contained seven (Rusché et al. 1997), thirteen (Madison-Antenucci et al. 1998) or twenty (Panigrahi et al. 2001a) proteins (in this discussion, we ignore polypeptides detected in these preparations that were later determined to be contaminants). A TAP-purified complex capable of pre-cleaved in vitro editing was also isolated from *L. tarentolae*, containing about fourteen proteins that all have orthologs in the *T. brucei* editosome (Aphasizhev et al. 2003c). Curiously, the *T. brucei* preparations containing seven and twenty proteins were both capable of a full-round of in vitro editing, which includes endonucleolytic cleavage, with comparable efficiency. This finding is especially strange considering the seven-protein preparation did not contain any recognizable endonucleases. One explanation for this inconsistency takes into account the results from Schnaufer et al. (2003) and Brecht et al. (2005). The TAP-tagging of either RNA editing ligases described in the former report isolated proteins that are present in all preparations of 20S editosomes capable of in vitro editing, including KRETA3. This polypeptide was recently shown in the latter report to have endonucleolytic activity. It is possible that KRETA3 may have supplied this activity to the different 20S editosome preparations for the in vitro editing assay. Another possibility is that an endonuclease that was present in amounts below detection conferred this activity in the smaller preparation.

Accessory factors: RNA binding proteins involved in editing

The participation of RNA binding proteins is an expected feature of RNA editing since it entails RNA:RNA hybridization and massive RNA processing. They are called accessory factors (Stuart et al. 2002) because of a supposed complementary role in U-insertion/deletion without being a permanent component of the 20S editosome (Panigrahi et al. 2001a, 2003a). A common strategy for isolation of these factors takes advantage of the RNA-binding activity by selecting mt proteins that bind to synthetic gRNAs (Köller et al. 1994) or poly(U) polymers (Leegwater et al. 1995; Bringaud et al. 1995; Hayman and Read 1999). It was logical to assume the existence of poly(U) binding proteins considering such stretches of sequence are present in edited transcripts and the 3′ oligo(U) tails of gRNAs. Thus far, five apparent RNA editing accessory factors have been identified: MRP1, MRP2, REAP1, RBP16 and TbRGG1. However, this approach has also resulted in a number of false positives. Examples of in vitro RNA binding proteins with no apparent role in RNA editing are *L. tarentolae* p18, which turned out to be the F_0F_1-ATPase subunit b (Speijer et al. 1997), and *L. tarentolae* glutamate dehydrogenase, both of which bound RNA for unknown reasons (Estévez et al. 1999), and *T. brucei* RNA binding protein RBP38, which may have a role in RNA stability (Sbicego et al. 2003).

The mitochondrial RNA-binding proteins MRP1 and MRP2 are associated in a heteromeric complex, which seems to have an as yet undefined role in RNA editing (Blom et al. 2001; Aphasizhev et al. 2003a; Vondrušková et al. 2005). After its identification by UV crosslinking to gRNAs (Köller et al. 1994), MRP1 was shown to have a role in editing (Köller et al. 1997). Moreover, it has in vitro matchmaking activity, facilitating the annealing of gRNA to its cognate mRNA (Müller et al. 2001). As a basic protein, MRP1 is thought to neutralize the negative charge of a bound gRNA, abolishing the potential repulsive forces from the incoming mRNA. The gRNA-mRNA duplex is believed to disassociate subsequently from the protein since it has low affinity for double stranded RNA (Müller et al. 2002). MRP1 has been implicated in editing because an anti-MRP1 antibody inhibits in vitro editing (Lambert et al. 1999) and co-immunoprecipitates editing activity (Allen et al. 1998) and gRNAs (Blom et al. 2001). However, RNA editing appeared to be only marginally affected in bloodstream *T. brucei* in which both alleles of the MRP1 gene were knocked out by homologous recombination, although these cells were not able to differentiate into the procyclic form (Lambert et al. 1999). MRP2 is much less studied. It has only little primary sequence similarity to MRP1, but shared features like similar hydropathy plots and a high pI indicate that these proteins have similar biochemical properties (Blom et al. 2001). RNAi knockdowns of either or both MRPs in procyclic *T. brucei* affected the editing and abundance of specific edited and never-edited mRNAs (Vondrušková et al. 2005). This pleimorphic phenotype, in conjunction with the impact on mt RNA abundance in the MRP1 bloodstream null mutant (Lambert et al. 1999), suggests that apart from a role in editing of specific transcripts, the MRPs may function in other forms of RNA processing (Simpson et al. 2004; Vondrušková et al. 2005). In addition, silencing of one MRP leads to the loss of the other, showing their stability is dependent on their joining together into a complex. A TAP-purified MRP complex from *L. tarentolae* is largely composed of both MRP proteins, with only substoichiometric amounts of three other proteins, provisionally named AP1-3. This complex was also observed to co-immunoprecipitate with the 20S editosome KRELs and gRNA processing KRET1 in an RNase-sensitive manner (Aphasizhev et al. 2003a).

TAP-purification of the *T. brucei* MRP complex shows that it is made up exclusively of the two MRPs (A. Zíková, K. Stuart, J. Lukeš, unpublished results).

RNA-editing associated protein 1 was purified by an original approach: to identify individual components, a panel of monoclonal antibodies was generated against a *T. brucei* editing complex that sediments at 35–40S (Madison-Antenucci et al. 1998 and see below). One of these antibodies bound to a 45 kDa protein that was immunolocalized to the mitochondrion and co-fractionated with TUTase and ligase activities. The antibody also inhibited in vitro editing activity. REAP-1 is an RNA binding protein, without similarity to known proteins or recognizable sequence motifs (Madison-Antenucci et al. 1998). Surprisingly, it does not even have an easily recognizable ortholog in *Leishmania* spp. (Simpson et al. 2003). A striking feature is a tandemly repeated region which is, however, not responsible for its RNA binding properties (Madison-Antenucci and Hajduk 2001). Because REAP-1 preferentially binds poly(G) RNA polymers in vitro and pre-edited mRNAs tend to be GC-rich, it was proposed to have a role in transporting pre-edited mRNAs to the editing complex. In vitro analysis of REAP1 binding to an assortment of RNA editing substrates showed that it has a much higher affinity for pre-edited mRNAs and pre-edited mRNA:gRNA duplexes than for edited mRNA:gRNA duplexes, edited mRNAs alone or never-edited transcripts (Madison-Antenucci and Hajduk 2001). Further work is required to substantiate this theory.

RBP16 was discovered by positive selection on a poly(U) column, followed by negative selection with poly(A) (Hayman and Read 1999). It is composed of a C-terminal cold-shock domain (CSD) and an N-terminal RGG domain, a hallmark of Y-box nucleic acid-binding proteins. The CSD is the main mediator of the interaction with gRNAs and a poorly characterized partner protein (Miller and Read 2003), a homolog to a human protein called p32, that potentially regulates RBP16 by stimulating its gRNA binding activity (Hayman et al. 2001). The RGG domain serves to enhance these interactions (Miller and Read 2003) and contains methylated arginines which may regulate protein function (Pelletier et al. 2001). RNAi-mediated silencing of RBP16 specifically reduced the editing of cyB mRNA, while only marginally affecting the editing of other mRNAs, suggesting a regulatory role in cyB expression (Pelletier and Read 2003). The stability of never-edited transcripts was also affected in the RBP16 knock-downs, which points to an additional, more general role in RNA metabolism for this protein. However, gRNA stability was not reduced, indicating that RBP16 regulates editing of cyB mRNA at the level of gRNA utilization. Yet Western blot analysis shows that RBP16 is expressed at similar levels in the bloodstream and procyclic forms, indicating that its differential expression is not the means of cyB editing regulation. Other possible mechanisms of regulation, such as interaction with its protein partner or post-translational modifications were not investigated (Pelletier et al. 2001; Hayman et al. 2001; Pelletier and Read 2003).

TbRGG1 was found during the screening of a *T. brucei* cDNA for a homolog to nucleolin (Vanhamme et al. 1998), which lead to a known protein that had been fished out of mt lysates by virtue of its ability to UV cross-link to poly(U) (Leegwater et al. 1995). TbRGG1 cross-hybridized the nucleolin probe because both proteins contain the so-called RGG domain, which is an established RNA binding motif (Burd and Dreyfuss 1994). A possible role in RNA editing was proposed for this protein because it co-localized with in vitro deletion activity, sedimenting at 35–40S in glycerol gradients of mt lysates. REAP-1 was also found to have a similar sedimentation profile, implying the two proteins are part of a larger complex in which various components associate with the 20S editosome (Madison-Antenucci et al. 1998). However, different results were reported by Vondrušková et al. (2005), who found that TbRGG1 sediments higher in the gradient, which would argue against such as association. Further work is obviously required to settle this issue. TbRGG1 should not be confused with another RGG protein which was identified in purified 20S editosomes. This protein (TbRGGm) is a homolog to a nuclear *T. cruzi* protein (Ouaissi et al. 2000), and is predicted to have a different molecular weight (Panigrahi et al. 2003b). Its role in the editing process, if any, is unknown.

The 20S versus the 40S editosome

RNA editing is a process in which many proteins participate, requiring the core enzymatic activities of the 20S editosome, which are at least in vitro only capable of performing single rounds of U-insertion/deletion, in concert with numerous other factors that together comprise the machinery that performs the editing of a complete mRNA (Simpson et al. 2004; Stuart et al. 2005). Clearly, non-20S editosome components are also important, such as the KRET1 TUTase complex, which generates gRNA oligo(U) tails, and likely also the RNA matchmaking MRP complex (Aphasizhev et al. 2003a; Vondrušková et al. 2005), both of which are proposed to be linked to the 20S editosome in an RNase sensitive manner (Aphasizhev et al. 2003c; Simpson et al. 2004). Initial efforts to resolve editing complexes were based on screening fractions from glycerol gradients of mt lysates for in vitro editing activity, chimera-forming or individual enzymatic activities involved in RNA editing. In *T. brucei*, two major peaks were found at 20S and 35–40S, respectively (Pollard et al. 1992; Corell et al. 1996). These results imply that the editosome may be a dynamic structure that is built around the core activities (Madison-Antenucci et al. 2002; Simpson et al. 2004). Support for a 'core' editosome as the central element of RNA editing is provided by the existence of a 20S complex in dyskinetoplastic cells, which is also capable of in vitro editing (Domingo et al. 2003). Although the

existence of a 40S editing complex in *T. brucei* is documented in the literature, it remains an enigmatic entity. Although in vitro editing sediments at 20S, no such peak is observed at 40S, despite the presence of TUTase, RNA ligase and chimera formation activities in 40S fraction (Pollard et al. 1992; Corell et al. 1996). In addition, there is an apparent absence of this complex in *L. tarentolae*, in which 10S and 25S peaks of in vitro editing were observed (Peris et al. 1994). The association of the MRP and KRET1 complexes with the editing complex does not significantly affect the sedimentation value of the *L. tarentolae* editing complex, since it sediments as 20S even upon RNase treatment (Aphasizhev et al. 2003a, c). This result implies if such an association also exists in *T. brucei*, it (this assocation by itself) alone cannot explain the presence of the 40S complex. Clearly, much more research is required to characterize what makes up a whole editosome.

Structure of gRNAs

Incorporation of gRNAs into the editing process could be problematic, in view of the multitude of primary sequences involved, not easily recognizable by the protein machinery. These molecules may overcome this problem by adopting a characteristic secondary structure (Schmid et al. 1995). Guide RNAs contain two intramolecular hairpin structures with single-stranded ends, while the oligo(U) tail is believed to adopt a helical structure. The two stem loops are believed to interact with each other via two As flanking the base of the shorter 5′ hairpin and a third at the apex of the 3′ hairpin. This creates a compact three-dimensional structure that may present distinctive patches of negative charge to interacting proteins such as MRP1 (Hermann et al. 1997). Furthermore, this structure has a very low thermodynamic stability, especially the 5′ hairpin which encompasses the anchor domain, allowing the gRNA to readily unfold and hybridize to its cognate mRNA (Schmid et al. 1995).

The 3′-hairpin of the gRNA, which envelops the information domain, is thought to persist upon initial hybridization with the mRNA (Leung and Koslowsky 1999, 2001a, b. The editosome is presented with a common structure, containing three duplexed regions: one between the anchor domain and its pre-mRNA binding site, the second one between the 3′ oligo(U) tail interacting and the purine-rich sequences 5′ of the ES and a third consisting of the remaining gRNA hairpin. It is proposed that this gRNA structure may also play a role in positioning the oligo(U) tail relative to mRNA sequences just upstream of the ES. The remaining hairpin is eventually melted as the editing proceeds on the mRNA because of its relatively low thermodynamic stability compared to the base pairing occurring between incoming Us and guiding residues.

The post-transcriptionally added 3′-oligo(U) tail is essential for RNA editing, as shown in vivo in KRET1-silenced cells (Aphasizhev et al. 2003a). In vitro experiments suggest that it acts to tether the 5′ mRNA fragment to the gRNA:mRNA duplex, since gRNAs without this region do not have properly re-ligated products, while other editing-associated events, such as chimera formation and endonucleolytic cleavage are undisturbed (Burgess et al. 1999). Interestingly, when U-tails are replaced by complementary sequences involving all four nucleotides, in vitro editing is enhanced, occurring without chimera formation (Burgess et al. 1999; Cruz-Reyes et al. 2001). However, such an interaction may be detrimental in vivo, since it may hamper melting of the duplex by helicases. In addition, the presence of poly(U) binding proteins, such as TbRGG1 (Leegwater et al. 1995; Vanhamme et al. 1998), may play a role in tethering this part of the gRNA to the mRNA (Burgess et al. 1999).

There is one gRNA that participates in the RNA editing process using an alternative mechanism, having a guide that is actually part of the 3′ UTR of cox2 mRNA (Kim et al. 1994; Golden and Hajduk 2005). This gRNA acts in *cis* through a bend that brings its information domain into proximity of the ES. Furthermore, this gRNA completely lacks the requisite structures needed to act in *trans* (Kapushoc and Simpson 1999; Golden and Hajduk 2005), indicating that its utilisation relies on different recognition mechanisms. Typical *trans*-acting gRNAs can be induced to act in *cis* by covalently linking them to their cognate pre-mRNA in vitro (Burgess et al. 1999). However, the discussed structure required for recognition by the editosome is still maintained, and thus this situation is not analogous to the *cis* acting gRNA of cox2.

Developmental regulation of RNA editing in *T. brucei*

As the *T. brucei* cryptogenes were being deciphered, a pattern of differential editing was discerned between the procyclic and bloodstream forms, suggesting a role for RNA editing in the regulation of gene expression in relation to the observed changes in energy metabolism (Stuart et al. 1997; Schnaufer et al. 2002). Most of the edited mRNAs that code for subunits of the NADH dehydrogenase (complex I) are preferentially edited in the bloodstream forms (Koslowsky et al. 1990; Souza et al. 1992, 1993). Unexpectedly, the subunit named ND7 actually has two editing blocks, the 5′ block being edited in both stages and the 3′ block only in the bloodstream stage (Koslowsky et al. 1990). Edited transcripts of the only ribosomal protein S12 (RPS12) encoded by the mt genome are more abundant at the bloodstream stage (Feagin and Stuart 1988) while there are more edited cyB mRNAs in procyclics (Read et al. 1992). The mechanism underlying differential editing in the two stages is unclear. The regulation of gRNA concentration would be a logical way of controlling RNA editing, but gRNA abundance remains constant throughout the life cycle of *T. brucei* (Koslowsky et al.

1992; Riley et al. 1994, 1995). It has been proposed that RNA editing is regulated at the level of gRNA utilization (Riley et al. 1995; Pelletier and Read 2003), but other factors such as stage-specific differential polyadenylation may play a role (Bhat et al. 1992; Kao and Read 2005; also see below). In order to further address this point, the use of in vitro editing system for bloodstream forms (Halbig et al. 2004) in conjunction with assays established for procyclics could be helpful. This approach has already uncovered subtle differences in the glycerol gradient distribution of editing activities between the two stages. Although differential RNA editing is well documented in *T. brucei*, it has not yet been observed in other digenetic trypanosomatids such as *T. cruzi* (Kim et al. 1994) or *L. tarentolae* (Carrillo et al. 2001).

Evolution of RNA editing

Shortly after its discovery, it has been proposed that RNA editing in kinetoplastids may be a relic of an ancient "RNA world" when only RNA molecules existed (Benne 1990). Although an interesting thought, the absence of autocatalytic activities, involvement of a number of rather mundane proteins and a possible elimination of editing in the course of evolution (see below) do not speak in favour of this idea.

So far, the U-insertion/deletion type of editing was also found in *T. borreli* (Lukeš et al. 1994; Maslov and Simpson 1994), a parasitic bodonid that may have diverged from trypanosomatids as early as 700 millions years ago (Fernandez et al. 1993). Editing also exists in the free-living *Bodo saltans* (Blom et al. 1998), which demonstrates that this process is not restricted to species with a parasitic lifestyle as suggested earlier (Borst 1993). Based on a comparative analysis of editing in various kinetoplastids, which revealed less editing in the crown species (Maslov et al. 1994), a retroposition model was proposed (Simpson and Maslov 1994; Simpson et al. 2000). It postulates a gradual elimination of editing as a consequence of reverse transcriptase activity that converts (partially) edited mRNAs into cDNAs, which by homologous recombination replace the original (pan-)edited cryptogenes. Given the extreme energetic demands and sloppiness of the process, editing "on-the-way-out" seems to be the more likely evolutionary pathway. Yet again, as so often with kinetoplastid editing, things are more complicated than anticipated. The cox2 transcript is unedited in *T. borreli* and edited in the trypanosomatids (Lukeš et al. 1994), which seems to suggest that for this transcript editing is "on-the-way-in". The cox2 mRNA editing domain is very small however, indicating that cox2 editing could have arisen relatively recently, presumably to compensate for genomic frameshift mutations, by making use of the already existing editing machinery and a *cis*-acting gRNA sequence (Blom et al. 1998). It is clear nonetheless, that kinetoplastid RNA editing is at least as ancient as the group itself. Investigation of other Euglenozoa may shed additional light on this problem.

Nevertheless, the driving force behind the origin and evolutionary maintenance of the process remains a mystery. Why did evolution produce such a seemingly unnecessary and cumbersome process, as it produces mt mRNAs in other eukaryotes with significantly less effort? It has been proposed that editing may provide kinetoplastids with advantageous qualities, such as: (1) extra level of regulation of mt gene expression (Stuart 1997); (2) accelerated evolution by creating more genetic variation (Landweber and Gilbert 1993); (3) multiple proteins coded by one gene (Read et al. 1994); (4) fixing mutations that have accumulated in the mt genome when the flagellates lived in an anaerobic environment (Cavalier-Smith 1997). Albeit not refuted, evidence close to none is available in favour of any of these scenarios. Moreover, the origin of editing may have nothing to do with its current biological role, if any. A scenario proposed earlier (Covello and Gray 1993) describes a neutral process, wherein the pre-existing enzymatic machinery, capable of U-insertions/deletions but performing a different set of functions, evolved into an indispensable RNA editing system after accumulation of the first mutations affecting T residues in kDNA. Once this system is established, it would allow for accumulation of additional mutations, thus making RNA editing essentially a selfish mechanism. All in all, the raison-d'etre of RNA editing remains anybody's guess.

Integration of RNA editing with other mt mRNA maturation processes

RNA editing is a process that is essential for the maturation of a significant portion of mt mRNAs. This phenomenon may be integrated into other RNA processing pathways, since it is shown to occur simultaneously with cleavage of polycistronic pre-mRNAs (Koslowsky and Yahampath 1997) and polyadenylation of these transcripts (Militello and Read 1999; Bessolitsina et al. 2005). In addition, the 20S editosome may also be coupled with processing of polycistronic gRNAs (Grams et al. 2000), although KRET1, the TUTase required for gRNA processing, has not been identified in the purified complexes (Ernst et al. 2003). The potential of the 20S complex to engage in diverse cleavage events is another explanation for the numerous resident putative endonucleases (Stuart et al. 2005). Some components of the editing machinery also seem to play a role in mRNA stability, such as RBP16 (Pelletier and Read 2003), the MRP complex (Vondrušková et al. 2005) and KRET1 (Ryan and Read 2005 and see below). Also the mt 9S and 12S rRNAs have post-transcriptionally added 3′oligo(U) tails (Adler et al. 1991), which hints at a possible role for one of the TUTases (Stuart et al. 2005). However, 12S rRNAs contain oligo(U) tails of heterogeneous length, suggesting the involvement of KRET1, while the 9S always bears a tail of eleven Us, implying

the involvement of a more stringent TUTase activity. The processing and stability of kRNAs will be discussed in the next section.

Other forms of mt mRNA processing

Transcription in kinetoplastid mitochondria results in the generation of polycistronic transcripts from both maxicircles and minicircles. *T. brucei* minicircles contain three or four potential gRNA transcription units, each flanked by short inverted repeats which have been proposed to function in minicircle transcription (Pollard et al. 1990). The transcription of gRNA gene initiates at its 5′ end and a polycistronic gRNA is transcribed. The polycistron is processed by a 19S RNA processing complex to cleave them into monocistronic gRNAs (Grams et al. 2000).

Maxicircles are also transcribed polycistronically. However, observations from *L. tarentolae* and *C. fasciculata* indicate that not all maxicircle genes are transcribed into polycistrons, since numerous gRNAs located within the intergenic regions are primary transcripts (Blum et al. 1990; van der Spek et al. 1991). In contrast, *T. brucei* maxicircles probably encode only three gRNAs and two of these gRNA genes are located within mRNA coding sequence (Clement et al. 2004). Polycistronic RNAs are processed into monocistronic RNAs by precise cleavage events. This processing includes precise cleavage events as well as polyadenylation and (for some mRNAs) editing. For transcripts in which a gRNA is supplied in *trans*, the formation of a proper 3′ end was shown to be editing-independent. Counterintuitively, editing may even precede the cleavage and polyadenylation events. The interaction of these activities with the 5′ cleavage activity may be what determines which mRNA will be translated. In contrast, analysis of a precursor edited by a gRNA in *cis* showed another chain of events with the secondary and tertiary structures of the 3′ end being important for the editing process (Koslowsky et al. 1997).

A significant fraction of the steady state mt mRNA population in *T. brucei* has a poly(A) tail (Militello and Read 1999). The 3′ poly(A) tail plays a critical role in modulating RNA stability, this effect however, varies dramatically among organisms and the subcellular compartment in which it occurs. In plant mitochondria, chloroplast and bacteria, polyadenylation has been reported to destabilize mRNAs (Carpousis et al. 1999; Temperley et al. 2003). Polyadenylation in kinetoplastid mitochondria is a complex and poorly understood process in which the length of the poly(A) track is partially dependent on the editing status of the RNA. Unedited mRNAs harbour only short poly$(A)_{20}$ tails, whereas mRNAs that are partly or fully edited have both short poly$(A)_{20}$ and long poly$(A)_{120–200}$ extensions (Read et al. 1994; Militello and Read 1999). In addition, stage-specific differences in the poly(A) tail length suggest that polyadenylation may regulate mt gene expression during the life cycle (Read et al. 1992, 1994; Bhat et al. 1992).

Polyadenylation seems to play a dual role in RNA stability in trypanosome mitochondria. In vitro RNA turnover studies demonstrate that unedited mRNAs with a poly$(A)_{20}$ tail are rapidly degraded compared to their non-adenylated counterparts (Ryan et al. 2003). Conversely, in the absence of a poly(A) tail, edited mRNAs degrade much more rapidly than their unedited counterparts. This rapid turnover also occurs on partially edited RNAs, thereby defining a small *cis*-acting edited element that facilitates RNA decay. Most surprisingly, while a poly$(A)_{20}$ tail stimulated decay of an unedited mt transcript, it impeded degradation of an edited RNA. Thus, a short edited region is sufficient to switch a poly$(A)_{20}$ tail from a destabilizing into a stabilizing element (Kao and Read 2005). From an energetic standpoint, it is presumably efficient for the cell to stabilize RNAs that have begun the editing process, so they can go on to be completely edited and translated. Such a postulate is supported by the observation that the proportion of polyadenylated molecules grow with the extent of editing (Militello and Read 1999). The opposing effect of polyadenylation on the stability of unedited and edited RNAs suggests a novel and unique regulation of RNA turnover (Kao and Read 2005).

Two distinct RNA turnover pathways that differ in degradation kinetics, nucleotide requirements and substrate specificity represent another unusual feature of the trypanosome mitochondrion. A UTP-independent pathway is responsible for a slow degradation of mRNA and does not require UTP or a mRNA poly(A) tail. A second pathway depends on the addition of UTP and rapidly degrades only poly(A) bearing mRNA. RNAi-depletion for KRET1 revealed its role in the UTP-stimulated turnover of poly(A) RNAs, as KRET1 probably uses UTP to polymerize Us on the 3′ end of mRNAs which then acts in *cis* as a signal for rapid decay (Militello and Read 2000; Ryan and Read 2005).

As exptected, in *T. brucei* poly(A)-specific degradation of kRNA proceeds in the 3′ to 5′ direction (Ryan et al. 2003). Recently, a mt hydrolytic exoribonuclease activity has been described in yeast that preferentially degrades poly(A) RNAs. This so-called mt degradosome plays a number of roles in stability and processing of mt RNA (Margossian et al. 1996). It is a two-protein complex composed of an exoribonuclease (DSS-1) and a RNA helicase (SUV3) (Dziembowski et al. 2003). Depletion by RNAi of the recently identified trypanosome homologue TbDSS-1 provides evidence that it affects the stability and editing of certain kRNAs (Penschow et al. 2004). The lack of exoribonuclease activity of the recombinant TbDSS-1 is consistent with its predicted dependence on an association with SUV3 for this functional activity. The *T. brucei* SUV3 homologue has not yet been analyzed. Based on data mining and preliminary results, the mt degradosome of trypanosomes resembles that of the yeast (Penschow et al. 2004).

Although RNA editing was discovered in 1986, it took 15 years to prove the physical existence of the proteins encoded by the edited mRNAs (Horváth et al. 2000a). The first, albeit indirect evidence came from experiments with *L. tarentolae* cells resistant to antimycin, the inhibitor of respiration. This type of resistance is caused by point mutations in the kDNA-encoded cyB gene, which mRNA is edited in its 5′ end. Truly, the mutated cyB gene was found in the resistant *L. tarentolae* cell line and its protein product was responsible for the antimycin resistance (Schnaufer et al. 2000). More recently, direct sequencing of proteins specified by the unedited and edited mRNAs provided compelling evidence of mt translation (Horváth et al. 2000a, b). Moreover, organellor labelling with ^{35}S-methionine revealed unique properties of mt proteins in the kinetoplastid mitochondrion (Tittawella 1998; Horváth et al. 2002). Difficulties with identifying the de novo synthesized proteins were caused in part by a high hydrophobicity and also the lack of sensitivity to the usual inhibitors of prokaryotic translation, such as chloramphenicol. A possible explanation for the latter phenomenon may rest in the unusual structure of the kinetoplast ribosome. Several domains and stem-loop structures are entirely missing from the kinetoplastid 9S and 12S mitoribosomal RNAs, which are the smallest among their counterparts (de la Cruz et al. 1985; Sloof et al. 1985). In detergent mt lysates, both rRNAs appear to be present in several large ribonucleoprotein complexes, sedimenting between 45S and 66S. As visualized by electron microscopy, the kinetoplastid mitoribosome retains the shape reminiscent of bacterial and other eukaryotic mt ribosomes (D.A. Maslov, personal communication).

Another unorthodox feature of the trypanosome mt genetic system is tRNA import. Unlike in most other organisms, all mt tRNAs are imported from the cytosol and are therefore of the eukaryotic type (Hancock and Hajduk 1990; Schneider and Maréchal-Drouard 2000). Interestingly, mt translation of trypanosomatids, representing one of the most derived prokaryotic-type translation systems has to exclusively utilize eukaryotic-type tRNAs imported from the cytosol (Tan et al. 2002). All in all, regulation of gene expression in the kinetoplastid organelle appears to occur at all levels of RNA processing. To obtain a complete picture of mt gene expression and its regulation, we will have to understand not only the mechanisms of each of the different maturation processes, but also the way they interact.

Conclusions and outlook

The last decade witnessed an impressive progress in our understanding of functions of mt proteins in *T. brucei*. It appears that its mitochondrion and that of the related flagellates could become the record–holder in the sheer number of proteins imported into it. Thanks to the methods of reverse genetics and proteomics, we may soon learn the function of all of these proteins. The flow of information from kDNA via kRNA to the handful of mt-encoded proteins apparently requires an involvement of multitude of proteins by far exceeding the set needed for equivalent processes in other mitochondria. With only a very few exceptions, this protein machinery is coded by the nuclear genome and offen appears to often perform functions similar to those mediated by homologous proteins in the cytoplasm. Thus, proteins traditionally operating in the cytoplasm (and nucleus) of a eukaryotic cell appear to have been hijacked by the organelle (Stuart et al. 2005).

Following the recent completion of whole genomes of *T. brucei* (Berriman et al. 2005), *T. cruzi* (El-Sayed et al. 2005) and *L. major* (Ivens et al. 2005), the establishment of whole mt proteome of these human pathogens represents the next logical step. Initial studies indicated the feasibility of such a task (Panigrahi et al. 2003), and functional analysis of identified proteins will inevitably follow. However, if the present trends of functional genomics in *T. brucei* prevail, individual proteins will be assayed only for their anticipated function. This leaves the studies of DNA replication, structure and segregation, RNA transcription, stability, editing, and translation, oxidative phosphorylation, ATP production, iron-sulfur biogenesis etc. virtually isolated from each other, despite the fact that they deal with the very same organelle. As this would represent a substantial bottleneck, a battery of phenotypic assays for a number of mt functions will have to be developed. Only such an integrative approach shall provide us with a holistic insight into this most interesting organelle.

It is a joy to study the kinetoplastid mitochondrion, as it still represents a box of secrets and unique solutions. We shall have our eyes open for unprecedented processes such as exkinetoplastidy, since kinetoplastid flagellates are likely to surprise us in the future as they did many times in the past.

Acknowledgements We thank Rob Benne (University of Amsterdam) and Dmitri A. Maslov (University of California) for critical reading of the manuscript and Jana Fišáková for artistic talent. This work was supported by grants from the Grant Agency of the Czech Academy of Sciences 5022302 and Z60220518, the National Institutes of Health 5R03TW6445-2 and the Ministry of Education of the Czech Republic 6007665801.

References

Abraham JM, Feagin JE, Stuart K (1988) Characterization of cytochrome c oxidase III transcripts that are edited only in the 3′ region. Cell 55:267–272

Abu-Elneel K, Robinson DR, Drew ME, Englund PT, Shlomai J (2001) Intramitochondrial localization of universal minicircle sequence-binding protein, a trypanosomatid protein that binds kinetoplast minicircle replication origins. J Cell Biol 153:725–733

Adler BK, Harris ME, Bertrand KI, Hajduk SL (1991) Modification of *Trypanosoma brucei* mitochondrial rRNA by posttranscriptional 3′ polyuridine tail formation. Mol Cell Biol 11:5878–5884

Alfonzo JD, Thiemann OH, Simpson L (1998) Purification and characterization of MAR1—mitochondrial associated ribonuclease from *Leishmania tarentolae*. J Biol Chem 273:30003–30011

Anderson L et al (1981) Identification of mitochondrial proteins and some of their precursors in two-dimensional electrophoretic maps of human cells. Proc Natl Acad Sci USA 78:2407–2411

Aphasizhev R, Sbicego S, Peris M, Jang SH, Aphasizheva I, Simpson AM, Rivlin A, Simpson L (2002) Trypanosome mitochondrial 3′ terminal uridylyl transferase (TUTase): the key enzyme in U-insertion/deletion RNA editing. Cell 108:637–648

Aphasizhev R, Aphasizheva I, Nelson RE, Simpson L (2003a) 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

Aphasizhev R, Aphasizheva I, Simpson L (2003b) A tale of two TUTases. Proc Natl Acad Sci USA 100:10617–10622

Aphasizhev R, Aphasizheva I, Nelson RE, Gao GH, Simpson AM, Kang XD, Falick AM, Sbicego S, Simpson L (2003c) Isolation of a U-insertion/deletion editing complex from *Leishmania tarentolae* mitochondria. EMBO J 22:913–924

Aphasizhev R, Aphasizheva I, Simpson L (2004) Multiple terminal uridylyltransferases of trypanosomes. FEBS Lett 572:15–18

Aphasizheva I, Aphasizhev R, Simpson L (2004) RNA-editing terminal uridylyl transferase 1—Identification of functional domains by mutational analysis. J Biol Chem 279:24123–24130

Arts G-J, Sloof P, Benne R. (1995) A possible role for the guide RNA U-tail as a specificity determinant in formation of guide RNA-messenger RNA chimeras in mitochondrial extracts of *Crithidia fasciculata*. Mol Biochem Parasitol. 73:211–222

Avliyakulov NK, Lukeš J, Ray DS (2004) Mitochondrial histone-like DNA-binding proteins are essential for normal cell growth and mitochondrial function in *Crithidia fasciculata*. Eukaryot Cell 3:518–526

Bakalara N, Simpson AM, Simpson L (1989). The leishmania kinetoplast-mitochondrion contains terminal uridylyltransferase and RNA ligase activies. J Biol Chem 264:18679–18686

Basselin M, Badet-Denisot M-A, Robert-Gero M (1998) Modification of kinetoplast DNA minicircle composition in pentamidine-resistant *Leishmania*. Acta Trop 70:43–61

Beattie DS, Howton MM (1996) The presence of rotenone-sensitive NADH dehydrogenase in the long slender bloodstream and the procyclic forms of *Trypanosoma brucei brucei*. Eur J Biochem 241:888–894

Benne R, Van den Burg J, Brakenhoff JPJ, Sloof P, Van Boom JH, Tromp MC (1986) Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains 4 nucleotides that are not encoded in the DNA. Cell 46:819–826

Benne R (1990) RNA editing in trypanosomes—is there a message? Trends Genet 6:177–181

Berriman M, et al. (2005) The genome of the African trypanosome *Trypanosoma brucei*. Science 309:416–422

Bessolitsina EA, Fedyakov AV, Merzlyak EM, Kolesnikov AA (2005) The ATPase subunit 6 gene of *Leptomonas seymouri* (Trypanosomatidae) is transcribed and edited as a polycistronic mRNA. Mol Biol (Moscow) 39:53–57

Besteiro S, Barrett MP, Riviere L, Bringaud F (2005) Energy generation in insect stages of *Trypanosoma brucei*: metabolism in flux. Trends Parasitol 21:185–191

Bhat GJ, Koslowsky DJ, Feagin JE, Smiley BL, Stuart K (1990) An extensively edited mitochondrial transcript in kinetoplastids encodes a protein homologous to ATPse subunit 6. Cell 61:885–894

Bhat GJ, Souza AE, Feagin JE, Stuart K (1992) Transcript-specific developmental regulation of polyadenylation in *Trypanosoma brucei* mitochondria. Mol Biochem Parasitol 52:231–240

Birkenmeyer L, Sugisaki H, Ray DS (1987) Structural characterization of site-specific discontinuities associated with replication origins of minicircle DNA from *Crithidia fasciculata*. J Biol Chem 262:2384–2392

Blom D, de Haan A, van den Berg M, Sloof P, Jirků M, Lukeš J, Benne R (1998) RNA editing in the free-living bodonid *Bodo saltans*. Nucleic Acids Res 26:1205–1213

Blom D, de Haan A, van den Burg J, van den Berg M, Sloof P, Jirků M, Lukeš J, Benne R (2000) Mitochondrial minicircles in the free-living bodonid *Bodo saltans* contain two gRNA gene cassettes and are not found in large networks. RNA 6:1–15

Blom D, van den Burg J, Breek CKD, Speijer D, Muijsers AO, Benne R (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

Blum B, Bakalara N, Simpson L (1990) A model for RNA editing in kinetoplastid mitochondria: guide RNA molecules transcribed from maxicircle DNA provide the edited information. Cell 60:198–198

Blum B, Sturm NR, Simpson AM, Simpson L (1991) Chimeric gRNA messenger-RNA molecules with oligo(U) tails covalently linked at sites of RNA editing suggest that U-addition occurs by transesterification. Cell 65:543–550

Blum B, Simpson L (1992) Formation of guide RNA messenger-RNA chimeric molecules in vitro, the initial step of RNA editing, is dependent on an anchor sequence. Proc Natl Acad Sci USA 89:11944–11948

Bochud-Allemann N, Schneider A (2002) Mitochondrial substrate level phosphorylation is essential for growth of procyclic *Trypanosoma brucei*. J Biol Chem 277:32849–32854

Borst P (1991) Why kinetoplast DNA networks? Trends Genet 7:139–141

Brecht M, Niemann M, Schulter E, Müller UF, Stuart K, Göringer HU (2005) TbMP42, a protein component of the RNA editing complex in African trypanosomes, has endo-exoribonuclease activity. Mol Cell 17:621–630

Bringaud F, Peris M, Zen KH, Simpson L (1995) Characterization of two nuclear-encoded protein components of mitochondrial ribonucleoprotein complexes from *Leishmania tarentolae*. Mol Biochem Parasitol 71:65–79

Burd CG, Dreyfuss G (1994) Conserved structures and diversity of functions of RNA-binding proteins. Science 265:615–21

Burgess MLK, Stuart K (2000) Sequence bias in edited kinetoplastid RNAs. RNA 6:1492–1497

Burgess MLK, Heidmann S, Stuart K (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

Carpousis AJ, Vanzo NF, Raynal LC (1999) mRNA degradation. A tale of poly(A) and multiprotein machines. Trends Genet 15:24–28

Carrillo R, Thiemann OH, Alfonzo JD, Simpson L (2001) Uridine insertion/deletion RNA editing in *Leishmania tarentolae* mitochondria shows cell cycle dependence. Mol Biochem Parasitol 113:175–181

Cavalier-Smith T (1997) Cell and genome coevolution: facultative anaerobiosis, glycosomes and kinetoplastan RNA editing. Trends Genet 13:6–9

Cech TR (1991) RNA editing: world's smallest introns? Cell 64:667–669

Chaudhuri M, Ajayi W, Hill GC (1998) Biochemical and molecular properties of the *Trypanosoma brucei* alternative oxidase. Mol Biochem Parasitol 95:53–68

Chen JH, Rauch CA, White JH, Englund PT, Cozzarelli NR (1995a) The topology of the kinetoplast DNA network. Cell 80:61–69

Chen JH, Rauch CA, White JH, Cozzarelli NR, Englund PT (1995b) Changes in network topology during the replication of kinetoplast DNA. EMBO J 14:6339–6347

Clement SL, Mingler MK, Koslowsky DJ (2004) An intragenic guide RNA location suggests a complex mechanism for mitochondrial gene expression in *Trypanosoma brucei*. Eukaryot Cell 3:862–869

Corell RA, Feagin JE, Riley GR, Strickland T, Guderian JA, Myler PJ, Stuart K (1993) *Trypanosoma brucei* minicircles en-

code multiple guide RNAs which can direct editing of extensively overlapping sequences. Nucleic Acids Res 21:4313–4320
Corell RA, Read LK, Riley GR, Nellissery JK, Allen TE, Kable ML, Wachal MD, Seiwert SD, Myler PJ, Stuart KD (1996) Complexes from *Trypanosoma brucei* that exhibit deletion editing and other editing-associated properties. Mol Cell Biol 16:1410–1418
Covello PS, Gray MW (1993) On the evolution of RNA editing. Trends Genet 9:265–268
Cruz-Reyes J, Rusché LN, Sollner-Webb B (1998) *Trypanosoma brucei* U-insertion and U-deletion activities co-purify with an enzymatic editing complex but are differentially optimized. Nucleic Acids Res 26:3634–3639
Cruz-Reyes J, Piller KJ, Rusché LN, Mukherjee M, Sollner-Webb B (1998a) Unexpected electrophoretic migration of RNA with different 3′ termini causes a RNA sizing ambiguity that can be resolved using nuclease P1-generated sequencing ladders. Biochemistry 37:6059–6064
Cruz-Reyes J, Rusché LN, Piller KJ, Sollner-Webb B (1998b) *Trypanosma brucei* RNA editing: adenosine nucleotides inversely affect U-deletion and U-insertion reactions at mRNA cleavage. Mol Cell 1:401–409
Cruz-Reyes J, Zhelonkina A, Rusché L, Sollner-Webb B (2001) Trypanosome RNA editing: simple guide RNA features enhance U-deletion 100-fold. Mol Cell Biol 21:884–892
Cruz-Reyes J, Zhelonkina AG, Huang CE, Sollner-Webb B (2002) Distinct functions of two RNA ligases in active *Trypanosoma brucei* RNA editing complexes. Mol Cell Biol 22:4652–4660
De la Cruz V, Lake JA, Simpson AM, Simpson L (1985) A minimal ribosomal-RNA-sequence and secondary structure of the 9S kinetoplast ribosomal-RNA from *Leishmania tarentolae*. Proc Natl Acad Sci USA 82:1401–1405
Decker CJ, Sollner-Webb B (1990) RNA editing involves indiscriminate U changes throughout precisely defined editing domains. Cell 61:1001–1011
Doležel D, Jirků M, Maslov DA, Lukeš J (2000) Phylogeny of the bodonid flagellates (Kinetoplastida) based on small subunit rRNA gene sequences. Int J Syst Evol Microbiol 50:1943–1951
Domingo GJ, Palazzo SS, Wang B, Pannicucci B, Salavati R, Stuart KD (2003) Dyskinetoplastic *Trypanosoma brucei* contains functional editing complexes. Eukaryot Cell 2:569–577
Downey N, Hines JC, Sinha KM, Ray DS (2005) Mitochondrial DNA ligases of *Trypanosoma brucei*. Eukaryot Cell 4:765–774
Drew ME, Englund PT (2001) Intramitochondrial location and dynamics of *Crithidia fasciculata* kinetoplast minicircle replication intermediates. J Cell Biol 153:735–743
Drozdz M, Palazzo SS, Salavati R, O'Rear J, Clayton C, Stuart K (2002) TbMP81 is required for RNA editing in *Trypanosoma brucei*. EMBO J 21:1791–1799
Dyková I, Fiala I, Lom J, Lukeš J (2003) *Perkinsiella amoebae*-like endosymbionts of *Neoparamoeba* spp., relatives of the kinetoplastid *Ichthyobodo*. Europ J Protistol 39:37–52
Dziembowski A, Piwowarski J, Hoser R, Minczuk M, Dmochowska A, Siep M, van der Spek H, Grivell L, Stepien PP (2003) The yeast mitochondrial degradosome, its composition, interplay between RNA helicase and RNAse activities and the role in mitochondrial RNA metabolism. J Biol Chem 278:1603–1611
El-Sayed NM, et al. (2005) The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. Science 309:409–415
Engel ML, Ray DS (1999) The kinetoplast structure-specific endonuclease I is related to the 5′ exo/endonuclease domain of bacterial DNA polymerase I and colocalizes with the kinetoplast topoisomerase II and DNA polymerase beta during replication. Proc Natl Acad Sci USA 96:8455–8460
Ernst NL, Panicucci B, Igo RP, Panigrahi AK, Salavati R, Stuart K (2003) TbMP57 is a 3′ terminal uridylyl transferase (TUTase) of the *Trypanosoma brucei* editosome. Mol Cell 11:1525–1536
Estévez AM, Kierszenbaum F, Wirtz E, Bringaud F, Grunstein J, Simpson L. (1999) Knockout of the glutamate dehydrogenase gene in bloodstream *Trypanosoma brucei* in culture has no effect on editing of mitochondrial mRNAs. Mol Biochem Parasitol. 100(1):5–17
Estévez AM, Simpson L (1999) Uridine insertion/deletion RNA editing in trypanosome mitochondria—a review. Gene 240:247–260
Feagin JE, Stuart K (1988) Developmental aspects of uridine addition within mitochondrial transcripts of *Trypanosoma brucei*. Mol Cell Biol 8:1259–1265
Feagin JE, Abraham JM, Stuart K (1988a) Extensive editing of the cytochrome *c* oxidase III transcript in *Trypanosoma brucei*. Cell 53:413–422
Feagin JE, Shaw JM, Simpson L, Stuart K (1988b) Creation of AUG initiation codons by addition of uridines within cytochrome *b* transcripts of kinetoplastids. Proc Natl Acad Sci USA 85:539–543
Fernandez AP, Nelson K, Beverly SM (1993) Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: perspective on the age and origins of parasitism. Proc Natl Acad Sci USA 90:11608–11612
Gao G, Kapushoc ST, Simpson AM, Thiemann OH, Simpson L (2001) Guide RNAs of the recently isolated LEM125 strain of *Leishmania tarentolae*: an unexpected complexity. RNA 7:1335–1347
Gao G, 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
Gao G, Simpson AM, Kang X, Rogers K, Nebohacova M, Li F, Simpson L (2005) Functional complementation of *Trypanosoma brucei* RNA in vitro editing with recombinant RNA ligase. Proc Natl Acad Sci USA 102:4712–4717
Gažiová I, Lukeš J (2003). Mitochondrial and nuclear localization of topoisomerase II in the flagellate *Bodo saltans* (Kinetoplastida), a species with non-catenated kinetoplast DNA. J Biol Chem 278:10900–10907
Golden DE, Hajduk SL (2005) The 3′-untranslated region of cytochrome oxidase II mRNA functions in RNA editing of African trypanosomes 'exclusively as a *cis* guide RNA. RNA 11:29–37
Grams J, McManus MT, Hajduk SL (2000) Processing of polycistronic guide RNAs is associated with RNA editing complexes in *Trypanosoma brucei*. EMBO J 19:5525–5532
Grams J, Morris JC, Drew ME, Wang ZF, Englund PT, Hajduk SL (2002) A trypanosome mitochondrial RNA polymerase is required for transcription and replication. J Biol Chem 277:16952–16959
Gray MW, Lang BF, Burger G (2004) Mitochondria of protists. Annu Rev Genet 38:477–524
Guilbride DL, Englund PT (1998) The replication mechanism of kinetoplast DNA networks in several trypanosomatid species. J Cell Sci 111:675–679
Hajduk SL, Klein VA, Englund PT (1984) Replication of kinetoplast DNA maxicircles. Cell 36:483–492
Halbig K, De Nova-Ocampo M, Cruz-Reyes J (2004) Complete cycles of bloodstream trypanosome RNA editing in vitro. RNA 10:914–920
Hancock K, Hajduk SL (1990) The mitochondrial tRNAs of *Trypanosoma brucei* are nuclear encoded. J Biol Chem 265:19208–19215
Harris ME, Hajduk SL (1992) Kinetoplastid RNA editing: in vitro formation of cytochrome b gRNA-mRNA chimeras from synthetic substrate RNAs. Cell 68:1091–1099
Hayman ML, Read LK (1999) *Trypanosoma brucei* RBP16 is a mitochondrial Y-box family protein with guide RNA binding activity. J Biol Chem 274:12067–12074
Hayman ML, Miller MM, Chandler DM, Goulah CC, Read LK (2001) The trypanosome homolog of human p32 interacts with RBP16 and stimulates its gRNA binding activity. Nucleic Acids Res 29:5216–5225
Hermann T, Schmid B, Heumann H, Göringer HU (1997) A three-dimensional working model for a guide RNA from *Trypanosoma brucei*. Nucleic Acids Res 25:2311–2318
Hernandez FR, Turrens JF (1998) Rotenone at high concentrations inhibits NADH-fumarate reductase and the mitochondrial

respiratory chain of *Trypanosoma brucei* and *T. cruzi*. Mol Biochem Parasitol 93:135–137
Horváth A, Neboháčová M, Lukeš J, Maslov DA (2002) Unusual polypeptide synthesis in the kinetoplast-mitochondria from *Leishmania tarentolae*—Identification of individual de novo translation products. J Biol Chem 277:7222–7230
Horváth A, Berry EA, Maslov DA (2000a) Translation of the edited mRNA for cytochrome b in trypanosome mitochondria. Science 287:1639–1640
Horváth A, Kingan TG, Maslov DA (2000b) Detection of the mitochondrially encoded cytochrome c oxidase subunit I in the trypanosomatid protozoan *Leishmania tarentolae*. Evidence for translation of unedited mRNA in the kinetoplast. J Biol Chem 275:17160–17165
Horváth A, Horáková E, Dunajčíková P, Verner Z, Pravdová E, Šlapetová I, Cuninková L, Lukeš J (2005) Down-regulation of the nuclear-encoded subunits of the complexes III and IV disrupts their respective complexes but not complex I in procyclic *Trypanosoma brucei*. Mol Microbiol 58:116–130
Huang CE, Cruz-Reyes J, Zhelonkina AG, O'Hearn S, Wirtz E, Sollner-Webb B (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
Huang CE, O'Hearn SF, Sollner-Webb B (2002) Assembly and function of the RNA editing complex in *Trypanosoma brucei* requires band III protein. Mol Cell Biol 22:3194–3203
Igo RP Jr, Palazzo SS, Burgess ML, Panigrahi AK, Stuart K (2000) Uridylate addition and RNA ligation contribute to the specificity of kinetoplastid insertion RNA editing. Mol Cell Biol 20:8447–8457
Igo RP Jr, Lawson SD, Stuart K (2002) RNA sequence and base pairing effects on insertion editing in *Trypanosoma brucei*. Mol Cell Biol 22:567–1576
Ivens AC et al (2005) The genome of the kinetoplastid parasite, *Leishmania* major. Science 309:436–442
Kable ML, Seiwert SD, Heidmann S, Stuart K (1996) RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA. Science 273:1189–1195
Kang XD, Falick AM, Nelson RE, Gao GH, Rogers K, Aphasizhev R, Simpson L (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
Kang XD, Rogers K, Gao GH, Falick AM, Zhou S, Simpson L (2005) Reconstitution of uridine-deletion precleaved RNA editing with two recombinant enzymes. Proc Natl Acad Sci USA 102:1017–1022
Kao CY, Read LK (2005) Opposing effect of polyadenylation on the stability of edited and unedited mitochondrial RNAs in *Trypanosoma brucei*. Mol Cell Biol 25:1634–1644
Kapushoc ST, Simpson L (1999) In vitro uridine insertion RNA editing mediated by *cis*-acting guide RNAs. RNA 5:656–669
Kim KS, Teixeira SM, Kirchhoff LV, Donelson JE (1994) Transcription and editing of cytochrome oxidase II RNAs in *Trypanosoma cruzi*. J Biol Chem 269:1206–1211
Klingbeil MM, Englund PT (2004) Closing the gaps in kinetoplast DNA network replication. Proc Natl Acad Sci USA 101:4333–4334
Klingbeil MM, Drew ME, Liu YN, Morris JC, Motyka SA, Saxowsky TT, Wang ZF, Englund PT (2001) Unlocking the secrets of trypanosome kinetoplast DNA network replication. Protist 152:255–262
Klingbeil MM, Motyka SA, Englund PT (2002) Multiple mitochondrial DNA polymerases in *Trypanosoma brucei*. Mol Cell 10:175–186
Köller J, Nörskau G, Paul AS, Stuart K, Göringer HU (1994) Different *Trypanosoma brucei* guide RNA molecules associate with an identical complement of mitochondrial proteins in vitro. Nucleic Acids Res 22:1988–1995
Köller J, Müller UF, Schmid B, Missel A, Kruft V, Stuart K, Göringer HU (1997) *Trypanosoma brucei* gBP21. An arginine-rich mitochondrial protein that binds to guide RNA with high affinity. J Biol Chem 272:3749–3757
Koslowsky DJ, Bhat GJ, Read LK, Stuart K (1991) Cycles of progressive realignment of gRNA with mRNA in RNA editing. Cell 67:537–546
Koslowsky DJ, Yahampath G (1997) Mitochondrial mRNA 3′ cleavage/polyadenylation and RNA editing in *Trypanosoma brucei* are independent events. Mol Biochem Parasitol 90:81–94
Koslowsky DJ, Bhat GJ, Perrolaz AL, Feagin JE, Stuart K (1990) The MURF3 gene of *Trypanosoma brucei* contains multiple domains of extensive editing and is homologous to a subunit of NADH dehydrogenase. Cell 62:901–911
Lambert L, Müller UF, Souza AE, Göringer HU (1999) The involvement of gRNA-binding protein gBP21 in RNA editing—an in vitro and in vivo analysis. Nucleic Acids Res 27:1429–1436
Landweber LF, Gilbert W (1993) RNA editing as a source of genetic variation. Nature 363:179–182
Lang BF, Burger G, O'Kelly CJ, Cedergren R, Golding GB, Lemieux C, Sankoff D, Turmel M, Gray MW (1997) An ancestral mitochondrial DNA resembling a eubacterial genome in miniature. Nature 387:493–497
Lee S-Y, Lee S-T, Chang K-P (1992) Transkinetoplastidy—a novel phenomenon involving bulk alterations of mitochondrion-kinetoplast DNA of a trypanosomatid protozoan. J Protozool 39:190–196
Leegwater P, Speijer D, Benne R (1995) Identification by UV cross-linking of oligo(U)-binding proteins in mitochondria of the insect trypanosomatid *Crithidia fasciculata*. Eur J Biochem 227:780–786
Leung SS, Koslowsky DJ (2001a) Interactions of mRNA and gRNAs involved in trypanosome mitochondrial RNA editing: structure probing of an mRNA bound to its cognate gRNA. RNA 7:1803–1816
Leung SS, Koslowsky DJ (2001b) RNA editing in *Trypanosoma brucei*: characterization of gRNA U-tail interactions with partially edited mRNA substrates. Nucleic Acids Res 29:703–709
Li C, Englund PT (1997) A mitochondrial DNA primase from the trypanosomatid *Crithidia fasciculata*. J Biol Chem 272:20878–20792
Linial M, Shlomai J (1987) Sequence-directed bent DNA helix is the specific binding site for *Crithidia fasciculata* nicking enzyme. Proc Natl Acad Sci USA 84:8205–8209
Liu B, Liu Y, Motyka SA, Agbo EEC, Englund PT (2005) Fellowship of the rings: the replication of kinetoplast DNA. Trends Parasitol 21:363–369
Lukeš J, Arts GJ, Van den Burg J, deHaan A, Opperdoes F, Sloof P, Benne R (1994) Novel pattern of editing regions in mitochondrial transcripts of the cryptobiid *Trypanoplasma borreli*. EMBO J 13:5086–5098
Lukeš J, Jirků M, Avliyakulov N, Benada O (1998) Pankinetoplast DNA structure in a primitive bodonid flagellate, *Cryptobia helicis*. EMBO J 17:838–846
Lukeš J, Hines JC, Evans CJ, Avliyakulov NK, Prabhu VP, Chen J, Ray DS (2001). Disruption of the *Crithidia fasciculata* KAP1 gene results in extensive structural rearrangement of the kinetoplast disc. Mol Biochem Parasitol 117:179–186
Lukeš J, Guilbride DL, Votýpka J, Zíková A, Benne R, Englund PT (2002). The kinetoplast DNA network: evolution of an improbable structure. Eukaryot Cell 1:495–502
Madison-Antenucci S, Hajduk SL (2001) RNA editing-associated protein 1 is an RNA binding protein with specificity for pre-edited mRNA. Mol Cell 7:879–886
Madison-Antenucci S, Sabatini RS, Pollard VW, Hajduk SL (1998) Kinetoplastid RNA-editing-associated protein 1 (REAP-1): a novel editing complex protein with repetitive domains. EMBO J 17:6368–6376
Madison-Antenucci S, Grams J, Hajduk SL (2002) Editing machines: the complexities of trypanosome RNA editing. Cell 108:435–438

Marande W, Lukeš J, Burger G (2005) Unique mitochondrial genome structure in diplonemids, the sister group of kinetoplastids. Eukaryot Cell 4:1137–1146

Margossian SP, Li H, Zassenhous HP, Butow RA (1996) The DexH box protein SUV3p is a component of a yeast mitochondrial 3′–5′ exoribonuclease that suppresses group I intron toxicity. Cell 84:199–209

Marini JC, Levene SD, Crothers DM, Englund PT (1982) Bent helical structure in kinetoplast DNA. Proc Natl Acad Sci USA 79:7664–7668

Maslov DA, Kolesnikov AA, Zaitseva GN (1984) Conservative and divergent base sequence regions in the maxicircle kinetoplast DNA of several trypanosomatid flagellates. Mol Biochem Parasitol 12:351–364

Maslov DA, Simpson L (1992) The polarity of editing within a multiple gRNA-mediated domain is due to formation of anchors for upstream gRNAs by downstream editing. Cell 70:459–467

Maslov DA, Simpson L (1994) RNA editing and mitochondrial genomic organization in the cryptobiid kinetoplastid protozoan *Trypanoplasma borreli*. Mol Cell Biol 14:8174–8182

Maslov DA, Zíková A, Kyselová I, Lukeš J (2002) A putative novel nuclear-encoded subunit of the cytochrome c oxidase complex in trypanosomatids. Mol Biochem Parasitol 125:113–25

McManus MT, Adler BK, Pollard VW , Hajduk SL (2000) *Trypanosoma brucei* guide RNA poly(U) tail formation is stabilized by cognate mRNA. Mol Cell Biol 20:883–891

Militello KT, Read LK (2000) UTP-dependent and independent pathway of mRNA turnover in *Trypanosoma brucei* mitochondria. Mol Cell Biol 20:2308–2316

Militello KT, Read LK (1999) Coordination of kRNA editing and polyadenylation in *Trypanosoma brucei* mitochondria: complete editing is not required for long poly(A) tract addition. Nucleic Acids Res 27:1377–1385

Miller MM, Read LK (2003) *Trypanosoma brucei*: functions of RBP16 cold shock and RGG domains in macromolecular interactions. Exp Parasitol 105:140–148

Missel A, Souza AE, Nörskau G, Göringer HU (1997) Disruption of a gene encoding a novel mitochondrial DEAD-box protein in *Trypanosoma brucei* affects edited mRNAs. Mol Cell Biol 17:4895–4903

Moreira D, López-Garcia P, Vickerman K (2004) An updated view of kinetoplastid phylogeny using environmental sequences and a closer outgroup: proposal for a new classification of the class Kinetoplastea. Int J Syst Evol Microbiol 54:1861–1875

Morris JC, Drew ME, Klingbeil MM, Motyka SA, Saxowsky TT, Wang ZF, Englund PT (2001) Replication of kinetoplast DNA: an update for the new millenium. Int J Parasitol 31:453–458

Müller UF, Lambert L, Göringer HU (2001) Annealing of RNA editing substrates facilitated by guide RNA-binding protein gBP21. EMBO J 20:1394–1404

Müller UF, Göringer HU (2002) Mechanism of the gBP21-mediated RNA/RNA annealing reaction: matchmaking and charge reduction. Nucleic Acids Res 30:447–455

Myler PJ, Glick D, Feagin JE, Morales TH, Stuart KD (1993) Structural organization of the maxicircle variable region of *Trypanosoma brucei*—identification of potential replication origins and topoisomerase II binding sites. Nucleic Acids Res 21:687–694

Nitz N, Gomes C, de Cassia Rosa A, D'Souza-Ault MR, Moreno F, Lauria-Pires L, Nascimento RJ, Teixeira ARL (2004) Heritable integration of kDNA minicircle sequences from *Trypanosoma cruzi* into the avian genome: insights into human Chagas disease. Cell 118:175–186

O'Hearn SF, Huang CE, Hemann M, Zhelonkina A, Sollner-Webb B (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

Ogbadoyi EO, Robinson DR, Gull K (2003) A high-order transmembrane structural linkage is responsible for mitochondrial genome positioning and segregation by flagellar basal bodies in trypanosomes. Mol Biol Cell 14:1769–1779

Onn I, Milman-Shtepel N, Shlomai J (2004) Redox potential regulates binding of universal minicircle sequence binding protein at the kinetoplast DNA replication origin. Eukaryot Cell 3:277–287

Ouaissi A, Vergnes B, Borges M, Guilvard E (2000) Identification and molecular characterization of two novel *Trypanosoma cruzi* genes encoding polypeptides sharing sequence motifs found in proteins involved in RNA editing reactions. Gene 253:271–280

Palazzo SS, Panigrahi AK, Igo RP, Salavati R, Stuart K (2003) Kinetoplastid RNA editing ligases: complex association, characterization, and substrate requirements. Mol Biochem Parasitol 127:161–167

Palmer JD (1997) Going, going, gone. Science 275:790–791

Panigrahi AK, Schnaufer A, Carmean N, Igo RP, Gygi SP, Ernst NL, Palazzo SS, Weston DS, Abersold R, Salavati R, Stuart KD (2001a) Four related proteins of the *Trypanosoma brucei* RNA editing complex. Mol Cell Biol 21:6833–6840

Panigrahi AK, Gygi SP, Ernst NL, Igo RP, Palazzo SS, Schnaufer A, Weston DS, Carmean N, Salavati R, Aebersold R, Stuart KD (2001b) Association of two novel proteins, TbMP52 and TbMP48, with the *Trypanosoma brucei* RNA editing complex. Mol Cell Biol 21:380–389

Panigrahi AK, Schnaufer A, Ernst NL, Wang B, Carmean N, Salavati R, Stuart K (2003a) Identification of novel components of *Trypanosoma brucei* editosomes. RNA 9:484–492

Panigrahi AK, Allen TE, Stuart K, Haynes PA, Gygi SP (2003b) Mass spectrometric analysis of the editosome and other multiprotein complexes in *Trypanosoma brucei*. J Am Soc Mass Spec 14:728–735

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

Penschow JL, Sleve DA, Ryan CM, Read LK (2004) TbDSS-1, an essential *Trypanosoma brucei* exoribonuclease homolog that has pleiotropic effects on mitochondrial RNA metabolism. Eukaryot Cell 3:1206–1216

Peris M, Frech GC, Simpson AM, Bringaud F, Byrne E, Bakker A, Simpson L (1994) Characterization of two classes of ribonucleoprotein complexes possibly involved in RNA editing from *Leishmania tarentolae* mitochondria. EMBO J 13:1664–1672

Piller KJ, Rusché LN, Cruz-Reyes J, Sollner-Webb B (1997) Resolution of the RNA editing gRNA-directed endonuclease from two other endonucleases of *Trypanosoma brucei* mitochondria. RNA 3:279–290

Pollard VW, Rohrer SP, Michelotti EF, Hancock K, Hajduk SL (1990) Organization of minicircle genes for guide RNAs in *Trypanosoma brucei*. Cell 63: 783–790

Pollard VW, Harris ME, Hajduk SL (1992) Native mRNA editing complexes from *Trypanosoma brucei* mitochondria. EMBO J 11:4429–4438

Rauch CA, Pérez-Morga D, Cozzarelli NR, Englund PT (1993) The absence of supercoiling in kinetoplast DNA minicircles. EMBO J 12:403–411

Ray DS (1989) Conserved sequence blocks in kinetoplast minicircles from diverse species of trypanosomes. Mol Cell Biol 9:1365–1367

Ray DS, Hines JC (1995) Disruption of the *Crithidia fasciculata* RNH1 gene results in the loss of two active forms of ribonuclease H. Nucleic Acids Res 23:2526–2530

Read LK, Myler PJ, Stuart K (1992) Extensive editing of both processed and preprocessed maxicircle CR6 transcripts in *Trypanosoma brucei*. J Biol Chem 267:1123–1128

Read LK, Stankey KA, Fish WR, Muthiani AM, Stuart K (1994) Developmental regulation of RNA editing and polyadenylation in four life cycle stages of *Trypanosoma congolense*. Mol Biochem Parasitol 68:197–206

Riley GR, Corepp RA, Stuart K (1994) Multiple guide RNAs for identical editing of *Trypanosoma brucei* apocytochrome b messenger RNA have an unusual minicircle location and are developmentally regulated. J Biol Chem 269:6101–6108

Riley GR, Myler PJ, Stuart K (1995) Quantitation of RNA editing substrates, products and potential intermediates—implications for developmental regulation. Nucleic Acids Res 23:708–712

Robinson KR, Beverley SM (2003) Improvements in transfection efficiency and tests of RNA interference (RNAi) approaches in the protozoan parasite *Leishmania*. Mol Biochem Parasitol 128:217–228

Rusché LN, Piller KJ, Sollner-Webb B (1995) Guide RNA-messenger-RNA chimeras, which are potential RNA editing intermediates, are formed by endonuclease and RNA ligase in trypanosome mitochondrial extract. Mol Cell Biol 15:2933–2941

Rusché LN, Cruz-Reyes J, Piller KJ, Sollner-Webb B (1997) Purification of a functional enzymatic editing complex from *Trypanosoma brucei* mitochondria. EMBO J 16:4069–4081

Rusché LN, Huang CE, Piller KJ, Hemann M, Wirtz E, Sollner-Webb B (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

Ryan CM, Read LK (2005) UTP-dependent turnover of *Trypanosoma brucei* mitochondrial mRNA requires UTP polymerization and involves the RET1 TUTase. RNA 11:1–11

Ryan CM, Militello KT, Read LK (2003) Polyadenylation regulates the stability of *Trypanosoma brucei* mitochondrial RNAs. J Biol Chem 278:32753–32762

Sabatini R, Hajduk SL (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

Salavati R, Panigrahi AK, Stuart KD (2001) Mitochondrial ribonuclease P activity of *Trypanosoma brucei*. Mol Biochem Parasitol 115:109–117

Salavati R, Panigrahi AK, Morach BA, Palazzo SS, Igo RP, Stuart K (2002) Endoribonuclease activities of *Trypanosoma brucei* mitochondria. Mol Biochem Parasitol 120:23–31

Saxowsky TT, Choudhary G, Klingbeil MM, Englund PT (2003) *Trypanosoma brucei* has two distinct mitochondrial DNA polymerase beta enzymes. J Biol Chem 278:49095–49101

Sbicego S, Alfonzo JD, Estévez AM, Rubio MA, Kang X, Turck CW, Peris M, Simpson L (2003) RBP38, a novel RNA-binding protein from trypanosomatid mitochondria, modulates RNA stability. Eukaryot Cell 2:560–568

Scheffler IE (1999) Mitochondria. Wiley-Liss, New York

Schmid B, Riley GR, Stuart K, Goringer HU (1995) The secondary structure of guide RNA molecules from *Trypanosoma brucei*. Nucleic Acids Res 23:3093–3102

Schnaufer A, Panigrahi AK, Panicucci B, Igo RP Jr, Wirtz E, Salavati R, Stuart K (2001) An RNA ligase essential for RNA editing and survival of the bloodstream form of *Trypanosoma brucei*. Science 291:2159–2162

Schnaufer A, Sbicego S, Blum B (2000) Antimycin A resistance in a mutant *Leishmania tarentolae* strain is correlated to a point mutation in the mitochondrial apocytochrome *b* gene. Curr Genet 37:234–241

Schnaufer A, Domingo GJ, Stuart K (2002) Natural and induced dyskinetoplastic trypanosomatids: how to live without mitochondrial DNA. Int J Parasitol 32:1071–1084

Schnaufer A, Ernst NL, Palazzo SS, O'Rear J, Salavati R, Stuart K (2003) Separate insertion and deletion subcomplexes of the *Trypanosoma brucei* RNA editing complex. Mol Cell 12:307–319

Schneider A, Marechal-Drouard L (2000) Mitochondrial tRNA import: are there distinct mechanisms? Trends Cell Biol 10:509–513

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

Seiwert SD, Heidmann S, Stuart K (1996) Direct visualization of uridylate deletion in vitro suggests a mechanism for kinetoplastid RNA editing. Cell 84:831–841

Shapiro TA (1993) Kinetoplast DNA maxicircles-networks within networks. Proc Natl Acad Sci USA 90:7809–7813

Shapiro TA, Englund PT (1995) The structure and replication of kinetoplast DNA. Annu Rev Microbiol 49:117–143

Shlomai J (2004) The structure and replication of kinetoplast DNA. Curr Mol Med 4:623–647

Simpson AGB, Lukeš J, Roger AJ (2002) The evolutionary history of kinetoplastids and their kinetoplast. Mol Biol Evol 19:2071–2083

Simpson AGB, Roger AJ (2004) Protein phylogenies robustly resolve the deep-level relationships within Euglenozoa. Mol Phylogenet Evol 30:201–212

Simpson L (1973) Structure and function of the kinetoplast DNA. J Protozool 20:2–8

Simpson L, Maslov DA (1994) RNA editing and the evolution of parasites. Science 264:1870–1871

Simpson L, Thiemann OH, Savill NJ, Alfonso JD, Maslov DA (2000) Evolution of RNA editing in trypanosome mitochondria. Proc Natl Acad Sci USA 97:6986–6993

Simpson L, Sbicego S, Aphasizhev R (2003) Uridine insertion/deletion RNA editing in trypanosome mitochondria: a complex business. RNA 9:265–276

Simpson L, Aphasizhev R, Gao G, Kang X (2004) Mitochondrial proteins and complexes in *Leishmania* and *Trypanosoma* involved in U-insertion/deletion RNA editing. RNA 10:159–170

Simpson L, Neckelmann N, de la Cruz VF, Simpson AM, Feagin JE, Jasmer DP, Stuart JE (1987) Comparison of the maxicircle (mitochondrial) genomes of *Leishmania tarentolae* and *Trypanosoma brucei* at the level of nucleotide sequence. Biol Chem. 262:6182–96

Sinha KM, Hines JC, Downey N, Ray DS (2004) Mitochondrial DNA ligase in *Crithidia fasciculata*. Proc Natl Acad Sci USA 101:4361–4366

Sloof P, De Haan A, Eier W, Van Iersel M, Boel E, Van Steeg H, Benne R (1992) The nucleotide sequence of the variable region in *Trypanosoma brucei* completes the sequence analysis of the maxicircle component of mitochondrial kinetoplast DNA. Mol Biochem Parasitol 56:289–299

Sloof P, Van den Burg J, Voogd A, Benne R, Agostinelli M, Borst P, Gutell R, Noller H (1985) Further characterization of the extremely small mitochondrial ribosomal RNAs from trypanosomes: a detailed comparison of the 9S and 12S RNAs from *Crithidia fasciculata* and *Trypanosoma brucei* with rRNAs from other organisms. Nucleic Acids Res 13:4171–4190

Souza AE, Myler PJ, Stuart K (1992) Maxicircle CR1 transcripts of *Trypanosoma brucei* are edited and developmentally regulated and encode a putative iron-sulfur protein homologous to an NADH dehydrogenase subunit. Mol Cell Biol 12:2100–2107

Souza AE, Shu HH, Read LK, Myler PJ, Stuart KD (1993) Extensive editing of CR2 maxicircle transcripts of *Trypanosoma brucei* predicts a protein with homology to a subunit of NADH dehydrogenase. Mol Cell Biol 13:6832–6840

Speijer D, Breek CK, Muijsers AO, Hartog AF, Berden JA, Albracht SP, Samyn B, Van Beeumen J, Benne R (1997) Characterization of the respiratory chain from cultured *Crithidia fasciculata*. Mol Biochem Parasitol 85:171–186

Stuart KD, Panigrahi AK (2002) RNA editing: complexity and complications. Mol Microbiol 45:591–596

Stuart KD, Feagin JE, Abraham JM (1989) The creation of nucleotide sequences in messenger RNA—a minireview. Gene 15:155–160

Stuart KD, Allen TE, Heidmann S, Seiwert SD (1997) RNA editing in kinetoplastid protozoa. Microbiol Mol Biol Rev 61:105–120

Stuart KD, Panigrahi AK, Schnaufer A, Drozdz M, Clayton C, Salavati R (2002) Composition of the editing complex of *Trypanosoma brucei*. Phil Trans R Soc Lond B Biol Sci 357:71–79

Stuart K, Salavati R, Igo RP, Lewis Ernst N, Palazzo SS, Wang B (2004) In vitro assays for kinetoplastid U-insertion-deletion editing and associated activities. Methods Mol Biol 265:251–272

Stuart KD, Schnaufer A, Ernst NL, Panigrahi AK (2005) Complex management: RNA editing in trypanosomes. Trens Biochem Sci 30:97–105

Sturm NR, Simpson L (1990a) Partially edited mRNAs for cytochrome *b* and subunit III of cytochrome oxidase from *Leishmania tarentolae* mitochondria: RNA editing intermediates. Cell 61:871–878

Sturm NR, Simpson L (1990b) Kinetoplast DNA minicircles encode guide RNAs for editing of cytochrome oxidase subunit III mRNA. Cell 61:879–884

Sturm NR, Maslov DA, Blum B, Simpson L (1992) Generation of unexpected editing patterns in *Leishmania tarentolae* mitochondrial mRNAs: misediting produced by misguiding. Cell 70:469–476

Tan TH, Pach R, Crausaz A, Ivens A, Schneider A (2002) tRNAs in *Trypanosoma brucei*: genomic organization, expression, and mitochondrial import. Mol Cell Biol 22:3707–3717

Temperley RJ, Seneca SH, Tonska K, Bartnik E, Bindoff LA, Lightowlers RN, Chrzanowska-Lightowlers ZM (2003) Investigation of a pathogenic mtDNA microdeletion reveals a translation-dependent deadenylation decay pathway in human mitochondria. Hum Mol Genet 12:2341–2348

Tittawella I (1998) Protein synthesis in mitochondria isolated from the trypanosomatid protozoan *Crithidia fasciculata*. FEBS Lett 423:351–356

Torri AF, Kunkel TA, Englund PT (1994) A beta-like DNA polymerase from the mitochondrion of the trypanosomatid *Crithidia fasciculata*. J Biol Chem 269:8165–8171

Ullu E, Tschudi C, Chakraborty T (2004) RNA interference in protozoan parasites. Cell Microbiol 6:509–519

Van der Spek H, Arts GJ, Zwaal RR, van den Burg J, Sloof P, Benne R (1991) Conserved genes encode guide RNAs in mitochondria of *Crithidia fasciculata*. EMBO J 10:1217–1224

Van Weelden SWH, Fast B, Vogt A, Van Der Meer P, Saas J, Van Hellemond JJ, Tielens AGM, Boshart M. (2003) Procyclic *Trypanosoma brucei* do not use Krebs cycle activity for energy generation. J Biol Chem 278:12854–12863

Vanhamme L, Pérez-Morga D, Marechal C, Speijer D, Lambert L, Geuskens M, Alexandre S, Ismaili N, Göringer U, Benne R, Pays E (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

Vickerman K (1990) The evolutionary expansion of the trypanosomatid flagellates. Int J Parasitol 20:1317–1331

Vickerman K (2000) Diplonemids. In: The illustrated guide to the protozoa, pp. 1157–1159

Vondrušková E, van den Burg J, Zíková A, Ernst NL, Stuart K, Benne R, Lukeš J (2005) 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 280:2429–2438

Wang Z, Englund PT (2001) RNA interference of a trypanosome topoisomerase II causes progressive loss of mitochondrial DNA. EMBO J 20:4674–4683

Wang BB, Ernst NL, Palazzo SS, Panigrahi AK, Salavati R, Stuart K (2003) TbMP44 is essential for RNA editing and structural integrity of the editosome in *Trypanosoma brucei*. Eukaryot Cell 2:578–587

Worthey EA, Schnaufer A, Mian IS, Stuart K, Salavati R (2003) Comparative analysis of editosome proteins in trypanosomatids. Nucleic Acids Res 31:6392–6408

Xu CW, Hines JC, Engel ML, Russell DG, Ray DS (1996) Nucleus-encoded histone H1-like proteins are associated with kinetoplast DNA in the trypanosomatid *Crithidia fasciculata*. Mol Cell Biol 16:564–576