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International Journal for Parasitology 31 (2001) 1403–1415

The Australian Society for Parasitology Inc.
INTERNATIONAL Journal for PARASITOLOGY

www.parasitology-online.com

Invited review

Unique aspects of mitochondrial biogenesis in trypanosomatids

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Received 14 May 2001; received in revised form 26 June 2001; accepted 28 June 2001

Abstract

Mitochondrial biogenesis consists of the sum of all processes required for the formation of the mitochondrial membranes as well as the soluble compartments they contain. Furthermore, it includes the replication of the mitochondrial genome and correct segregation of the organelles during cell division. Mitochondrial proteins come from two sources, a limited but essential set of inner membrane proteins is encoded by the mitochondrial genome, whereas the large majority (90–95%) is derived from nucleus-encoded genes and are posttranslationally imported into the organelle. Trypanosomatids belong to the earliest diverging branches of the eukaryotic evolutionary tree which have mitochondria. This is reflected in the organisation of their mitochondrial DNA that consists of a network of two classes of topologically interlocked circular DNA molecules as well as many unique features in their mitochondrial biogenesis. The proteins encoded on the mitochondrial genome are conventional for a mitochondrial genome, their expression, however, involves a complex series of processes. Many genes represent incomplete open reading frames and their primary transcripts have to remodelled by RNA editing to convert them into translatable mRNAs. RNA editing is mediated by small mitochondria-encoded transcripts, the guide RNAs, and is in that form specific for trypanosomatids and closely related organisms. Mitochondrial translation is also unconventional. No tRNA genes are encoded on the mitochondrial genome. Instead, mitochondrial protein synthesis functions exclusively with imported cytosolic, eukaryotic-type tRNAs. The composition of mitochondrial ribosomes is also unusual in that they contain the smallest known rRNAs. They are about 30% shorter than the already much reduced rRNAs in human mitochondria. Furthermore, the topological organisation of the mitochondrial genome requires an elaborate replication machinery involving topoisomerases. Finally, some trypanosomatids have life cycle stages exhibiting very different mitochondrial activities and can therefore serve as a model system for the regulation of mitochondrial biogenesis.

Keywords: Mitochondria; *Trypanosoma*; *Leishmania*; Kinetoplast; Mitochondrial translation; RNA editing; Mitochondrial RNA import

1. Introduction

There is overwhelming evidence for the endosymbiont theory which states that mitochondria originate from a fusion of a free-living prokaryote with a primitive, probably nucleated cell. The evolutionary history of eukaryotes and the emergence of mitochondria are therefore tightly linked, resulting in the fact that the big majority of all eukaryotes contain mitochondria (Scheffler, 1999). The few exceptions known, such as *Giardia*, *Entamoeba* and *Trichomonas* appear to have either a structure derived from mitochondria, the hydrogenosome, or to have lost the organelle secondarily (Dyall and Johnson, 2000). It is therefore not surprising that a large body of work has accumulated on structure, function and biogenesis of mitochondria. Much of that work was done in one of the experimentally most accessible eukaryotic systems, the yeast *Saccharomyces cerevisiae*. These studies led to the elucidation of novel biological principles and molecular mechanisms, many of which were shown to be valid for all eukaryotes (Neupert, 1997; Rassow and Pfanner, 2000). In this review, I will summarise mitochondrial biogenesis in trypanosomatids. The family *Trypanosomatidae* consists of a large group of flagellated parasitic protozoa that are responsible for diseases in humans and animals, such as human sleeping sickness and nagana of cattle in Africa (*Trypanosoma brucei* spp.) and Chagas disease in Latin America (*Trypanosoma cruzi*). It also includes *Leishmania* spp. which cause different forms of leishmaniasis in much of the tropical and subtropical world and *Crithidia* spp. which parasitise insects only. Even though much is already known about mitochondrial biogenesis from yeast, one should not forget that one of the main features of life is diversity, a fact often ignored by mainstream molecular biology. Indeed, while the well-studied mechanisms of mitochondrial biogenesis in yeast are essential, they are not sufficient to explain the formation of trypanosomatid mitochondria. Trypanosomatids belong

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0020-7519/01/$20.00
PII: S0020-7519(01)00296-X

to the earliest diverging branches of the eukaryotic evolutionary tree, which have bona fide mitochondria involved in oxidative phosphorylation (Sogin et al., 1986). This may explain why many features of mitochondrial biogenesis are unique for this group of organisms. Furthermore, it makes similarities of the process between trypanosomatids and other systems all the more meaningful as they are probably shared among all eukaryotes.

2. General aspects of mitochondrial biogenesis

Mitochondria can have very diverse structures, however, all have a double membrane and function in oxidative phosphorylation. Without a single exception, they have a genome (six to more than 2000 kb in size) encoding generally a limited number (13 in yeast and humans) of proteins and a translation system allowing the production of these proteins (Scheffler, 1999). However, sequencing of mitochondrial genomes from lower eukaryotes has shown that many more proteins can be encoded on the mitochondrial DNA. The most extreme example identified is *Reclinomonas americana* whose mitochondrial genome encodes more than 70 proteins (Gray et al., 1999). Mitochondrial biogenesis consists of the sum of all processes leading to the formation of the mitochondrial membranes as well as of the soluble compartments they encompass. Biological membranes, including mitochondrial ones, are not formed de novo but are derived from the growth of preexisting membranes (Voelker, 1991). Since there is not much known about this important aspect of mitochondrial biogenesis, it will not be further discussed here. The proteins of mitochondria come from two sources, in most cases more than 95% are encoded in the nucleus, synthesised in the cytosol and posttranslationally imported into mitochondria. A small but essential part is encoded and produced in the mitochondria. These generally specify components of the respiratory chain and the organellar translation machinery (Attardi and Schatz, 1988). In addition to proteins, the organellar translation machinery also needs rRNAs and tRNAs. In all cases known, the rRNAs are encoded by the mitochondrial genome. For tRNAs, the situation is different. The mitochondrial genome of higher metazoa encodes a complete set of tRNAs but there are organisms which lack a variable number of apparently essential mitochondrial tRNA genes. It has been shown that in these cases, tRNAs are imported from the cytosol (Schneider and Marechal-Drouard, 2000).

Besides the formation of the lipid bilayer of mitochondrial membranes, the main processes required for mitochondrial biogenesis are therefore import and intramitochondrial sorting of nucleus-encoded proteins and the translation of proteins within the organelle. Furthermore, during cell division, the mitochondrial genome has to be replicated and segregated to the newly formed organelles. All the above facts are valid for mitochondria from any source, including trypanosomatids. However, if we look at the formation of trypanosomatid mitochondria in more detail, some striking differences to other organisms emerge.

3. Trypanosomatid mitochondria

3.1. Morphology

In contrast to most eukaryotes, which have hundreds of individual mitochondria, trypanosomatids have a single mitochondrion only (Simpson, 1972). The most unusual structure in the organelle is its DNA (generally called the kinetoplast DNA: kDNA), which morphologically appears as a disc-like structure in the matrix. There is good evidence for a physical connection between the kDNA and the basal body of the cells single flagellum since a kDNA/basal body complex can be isolated even in the presence of detergent. Furthermore, in *T. brucei* it was possible to visualise a tripartite attachment complex consisting of a group of filaments connecting the basal body to the outer mitochondrial membrane and a second cluster of filaments inside mitochondria which attach the kDNA to the corresponding region of the inner membrane. As predicted, due to the need of a membrane potential for mitochondrial function, the two sets of filaments are separated by intact layers of the inner and outer membranes. At present, nothing is known about molecular components of this attachment zone (Gull, 1999).

3.2. Mitochondrial genome

The structure of parasite mitochondrial genomes including those of trypanosomatids has recently been excellently reviewed in this journal (Feagin, 2000). I will therefore only briefly summarise the main points. The kDNA shows one of the most amazing topological organisations for any DNA found in nature. It consists of two classes of circular molecules of different sizes, the maxi- and the minicircles. Approximately 50 copies of the maxicircle DNA (20–40 kb in size depending on the species) are found in each organelle. Minicircles are smaller (0.65–2.5 kb) and found in 5000–10 000 copies per organelle in most trypanosomatid species. Maxi- and minicircles are topologically interlocked, both among themselves, as well as between each other forming a huge network of about 10^7 kDa. The maxicircles that contribute about 10% of the mass of the network are structurally and functionally analogous to the mitochondrial DNA of other organisms (Shapiro and Englund, 1995). In *T. brucei* and *Leishmania tarentolae* they encode 13 proteins of known identity. These are cytochrome *b* of the bc1-complex (CY*b*), subunits I–III of cytochrome oxidase (COI–III), subunit 6 of the adenosine triphosphatase (ATPase; A6), six subunits of the reduced nicotinamide adenine dinucleotide- (NADH)-dehydrogenase (ND1, 4, 5, 7–9) and a ribosomal protein (S12). Furthermore, five open reading frames (ORFs) of unknown function were also found, some of which may

encode for further components of the NADH dehydrogenase. Although the complement of maxicircle genes is conventional for a mitochondrial genome, the structure of some of these genes are very unusual. They are termed cryptogenes, which means that their transcripts have to be remodelled by gRNA-mediated RNA editing in order to convert them into translatable substrates (see Section 4.1). The genetic information necessary for RNA editing is specified by short transcripts called gRNAs, which are mainly encoded on the minicircle DNA. In addition to the 18 proteins encoded on the maxicircle DNA, one also finds the genes for the ssrRNA and the lsrRNAs. Genes for tRNAs, on the other hand, are completely absent in the mitochondrial genome of trypanosomatids. All tRNAs necessary for translation are imported from the cytosol. Approximately 90% of the kDNA network mass is due to minicircle DNA. In contrast to maxicircles, they are heterogeneous in sequence and encode the majority of gRNAs. This is reflected by the fact that in different trypanosomatid species there is a good correlation between number of distinct minicircle classes and extent of RNA editing.

3.3. Overview of trypanosomatid mitochondrial biogenesis

The number of mitochondria-encoded proteins is small (about 18) in trypanosomatids which is typical for most mitochondria. Expression of these proteins, however, requires processes which are either unique for trypanosomes or show significant differences to other organisms. In addition to the RNA editing mentioned above, mitochondrial translation also shows some unique features. The composition of the ribosomes is unusual in that they have only very short rRNAs. In addition to that, they have to function exclusively with imported eukaryotic-type tRNAs. When the mitochondrion divides, further problems arise since the complex network of its bipartite genome has to be duplicated and the correct topology of the newly synthesised genome has to be established. Finally, some trypanosomatids have life cycle stages exhibiting very different mitochondrial activities. Bloodstream *T. brucei* cells have mitochondria whose functions are repressed and which cannot perform oxidative phosphorylation. After differentiation into the insect stage procyclic form, however, there is a boost in mitochondrial biogenesis. The question of how this transition of mitochondrial biogenesis is regulated and what role it plays in the differentiation process in general is clearly of great interest. The processes listed in this section are of special importance for mitochondrial biogenesis in trypanosomatids and will now be discussed in more detail.

4. Mitochondrial gene expression

4.1. RNA editing

Many maxicircle transcripts derived from cryptogenes generally do not encode for complete ORFs and they are not necessarily collinear with their mature mRNAs. In contrast to their precursors, the mature mRNAs encode ORFs homologous to mitochondrial proteins of other species. The process responsible for the conversion of the primary transcripts into mature mRNAs is called RNA editing and consists of the insertion and/or deletion of a variable but defined number of uridine residues at specific positions in the precursor transcripts. The first examples of RNA editing were discovered in trypanosomatids 15 years ago. Only later was it recognised that various forms of RNA editing are quite widespread in organelles and can even occur in the nucleus. The gRNA-mediated form of RNA editing, however, remains specific for trypanosomatids and closely related organisms. Great effort has been invested to elucidate the mechanism and many of the initial questions have been answered. In this section, I intend to give a short summary and to discuss some recent developments in the field, for more detailed information the reader is referred to reviews, which were published recently (Sollner-Webb, 1996; Stuart et al., 1997; Estevez and Simpson, 1999).

The first breakthrough in the study of the RNA editing mechanism was the discovery of the guide RNAs (gRNAs) (Blum et al., 1990). They were the first known *trans*-acting factor required for RNA editing. The great significance of this discovery lies in the fact that such a bizarre process as RNA editing, which in some extreme cases is responsible for the apparent de novo synthesis of 60% of an mRNA (Feagin et al., 1988), is nevertheless governed by the well-known principle of nucleotide base pairing, albeit allowing non-conventional G–U pairs. gRNAs are small mini- and maxicircle-encoded transcripts and show three distinct regions. The 5′-part consists of an anchor domain hybridising to unedited or previously edited sequences of the corresponding substrate mRNA, a guiding region consisting of the central part of the molecule specifying the sequence of the region to be edited and finally a posttranscriptionally added poly U-tail. Detailed models of how gRNAs mediate RNA editing have been proposed but their discussion is beyond the scope of this review. In short, RNA editing can be summarised as a series of ordered chemical reactions. First, there is an endonucleolytic cleavage recognising the preedited region of the gRNA/mRNA duplex, the next reaction is the addition or removal of the correct number of uridine(s) at the specified editing site and finally the editing cycle is completed by the ligation of the edited 5′-half to the as yet unedited 3′-end of the mRNA. In the last few years, it has been possible using in vitro editing systems, to show that all of these reactions are catalysed by proteins as suggested by the ‘enzyme cascade model’ and not by the gRNA(s) itself as was proposed in the ‘transesterfication model’ (Hajduk et al., 1993). Both deletional and insertional-type RNA editing can be reconstituted in vitro and appear to be associated with large ribonucleoprotein (RNP)-complexes isolated from trypanosomatid mitochondria. The in vitro reactions were shown to be gRNA-dependent and to require ATP. For insertional-type editing uridine tripho-

sphate (UTP) was required indicating that the uridines inserted into the edited region derive from the soluble pool and not from the poly U-tail of the gRNA (Kable et al., 1996; Seiwert et al., 1996; Seiwert and Stuart, 1994).

RNA editing consists of a complex series of reactions and it is therefore not surprising that there is no agreement yet on the exact number of proteins present in the RNP editing complexes. It is, however, encouraging to see that a consensus is emerging concerning one catalytic component. Several laboratories have recently reported the characterisation of two mitochondrial proteins in *T. brucei* of 52 and 48 kDa in size (*Tb*REL1 and 2) which are adenylatable, exhibit RNA ligase activity and whose predicted amino acid sequences show ligase signatures (McManus et al., 2001; Panigrahi et al., 2001; Rusche et al., 2001; Schnaufer et al., 2001). In vivo depletion of the 52 kDa protein is lethal in procyclic trypanosomes and before the cells die, a marked reduction of RNA editing is observed, whereas the steady-state level of unedited transcripts remains unaffected. Interestingly, the protein appears also to be essential in bloodstream cells (Schnaufer et al., 2001). This is surprising at first since dyskinetoplastic mutants of *T. brucei* which carry large deletions in the mitochondrial genome or even lack it entirely can be maintained in the mammalian host, suggesting that in the bloodstream stage translation of mitochondrial protein is not essential (Stuart, 1971). On the other hand, it has been shown that complete editing of some mitochondrial transcripts encoding subunits of NADH-dehydrogenase (ND7,8) occurs in bloodstream forms only (Koslowsky et al., 1990; Souza et al., 1992). This suggests a role for mitochondrial translation in bloodstream trypanosomes, at least in wild-type cells. Alternatively, the essential nature of TbREL1 in bloodstream cells might be explained by an as yet unknown cytosolic function of the protein.

Other *T. brucei* proteins that might be involved in RNA editing have been described, though their role is less clear. These include a 21 kDa protein (gBP21) that binds gRNAs with high affinity and is associated with RNA editing complexes. The protein is able to accelerate the rate of mRNA/gRNA duplex formation, the presumed first step of RNA editing (Muller et al., 2001). Whereas in vitro evidence suggests that gBP21 is required for RNA editing gene knock-out experiments were unable to confirm its role in vivo. Another component RNA editing associated protein 1 (REAP-1) of the RNP editing complex has recently been characterised. It consists of a mitochondrial protein of 45 kDa showing an unusual 21 amino acid repeat region. Immunodepletion of the protein abolishes in vitro editing (Madison-Antenucci et al., 1998). Its in vivo role, however, remains to be investigated. Finally, it has been proposed that a mitochondrial DEAD-box RNA-helicase (mHel61p) is required for RNA editing in vivo. The protein might be involved in the resolution of the gRNA/mRNA duplex after completion of the editing reaction. Gene knock-out experiments resulted in a slow growth phenotype in procyclic trypanosomes. More importantly, a reduction of the level of edited mRNAs could be shown in these cells whereas the steady-state levels of unedited mRNAs were not affected (Missel et al., 1997). Extracts from the disruption strain, however, showed no phenotype when tested for in vitro editing.

Guide RNA-mediated RNA editing is being actively investigated by a number of research groups and much progress on the elucidation of the mechanism and on the characterization of the other catalytic activities can be therefore expected in the near future. In contrast, as discussed in the Section 4.2, we still know very little on mitochondrial translation in trypanosomatids.

4.2. Mitochondrial translation

The function of mitochondrial RNA editing is to produce translatable mRNAs. Despite much effort it has only recently been possible to show that translation occurs in trypanosomatid mitochondria. The first convincing, though indirect evidence was presented by an elegant experiment in *L. tarentolae* (Schnaufer et al., 2000). The approach was to select for *L. tarentolae* cells that were resistant to the respiratory inhibitor, antimycin. Results in other organisms had shown that this type of resistance is caused by point mutations in two evolutionary conserved regions of the mitochondria-encoded cytochrome *b* gene. Indeed, a point mutation in the corresponding region of the cytochrome *b* gene was also found in the resistant *L. tarentolae* cell line and it was concluded that expression of the mutated gene product was responsible for the antimycin resistance. Since the 5′-part of the cytochrome *b* transcript is edited, the experiment provided evidence not just for translation in general but for translation of an edited message. More recently, isolation of respiratory complexes followed by two dimensional gel-electrophoresis allowed direct sequencing of two peptides from mitochondria-encoded proteins, one derived from cytochrome *b* (Horvath et al., 2000a) and the other from cytochrome oxidase subunit I (Horvath et al., 2000b). Both peptides showed the sequences predicted from their corresponding mitochondrial genes, if editing of the cytochrome *b* message was taken into account. It was also possible to detect mitochondrial translation by in organelle labelling with ^{35}S-methionine. In isolated mitochondria of *Crithidia fasciculata*, a limited set of proteins could be labelled and the observed translation was, as expected, insensitive towards the cytosol-specific translation inhibitor, cycloheximide (Tittawella, 1998). Interestingly, however, it was also resistant to the prokaryotic-type translation inhibitors chloramphenicol and erythromycin. An analogous experiment in *T. brucei* yielded similar results concerning sensitivity to cycloheximide, however, in this case sensitivity to chloramphenicol and erythromycin was observed (Nabholz et al., 1999). The latter finding agrees with an earlier report measuring a cycloheximide-resistant but chlorampenicol-sensitive translation activity in digitonin-permeabilised trypanosomes (Shu and Göringer, 1998).

Sensitivity to chloramphenicol is considered a hallmark for mitochondrial translation. However, the situation is controversial in trypanosomatids since the region in their mitochondrial 12S rRNA, which is homologous to the chloramphenicol-binding site of other organisms is only moderately conserved and based on these comparisons it was predicted that mitochondrial translation might be chloramphenicol-resistant (Eperon et al., 1983). The problem with the in organelle labelling experiments in trypanosomatids is that they are very inefficient. At least one to two orders of magnitude less signal is obtained than in comparable experiments with yeast mitochondria (Nabholz et al., 1999). In summary, the described experiments have shown that a translation system capable of functioning with edited and unedited mRNA exists in trypanosomatid mitochondria. It is, however, important and most likely very rewarding to analyse mitochondrial translation in trypanosomatids in more detail.

All ribosomes are composed of rRNAs and ribosomal proteins. Mitochondrial ribosomes are of the prokaryotic-type and their rRNAs have been minimised during evolution. Whereas the 16S rRNA and 23S rRNA in *Escherichia coli* are 1542 and 2904 nucleotides in length, the homologous molecules in human mitochondria have been reduced to a length of 953 and 1555 nucleotides. In trypanosomatids (e.g. *T. brucei*) this reduction is taken much further, the 9S rRNA is 611 and the 12S rRNA only 1150 nucleotides long (Eperon et al., 1983; delaCruz et al., 1985a,b; Sloof et al., 1985; Fig. 2). This makes them the shortest rRNAs known to date, with the possible exception of the rRNA fragments encoded on the apicomplexan mitochondrial genomes (Gillespie et al., 1999). The overall similarity of trypanosomatid mitochondrial rRNAs with prokaryotic rRNAs is low. However, short sequence elements as well as secondary structures that are universally conserved in all known rRNAs are found. Most domains of prokaryotic rRNAs have been retained though some stems and loops have been drastically reduced or completely eliminated. Interestingly, the most conserved region on the 12S rRNA of trypanosomes corresponds to the peptidyl-transferase centre in the prokaryotic ls rRNA. Despite their short rRNAs, trypanosomatid mitochondrial ribosomes are clearly functional and therefore may serve as a model to investigate the essential function of rRNA in general. There is not much known about ribosomal proteins in trypanosomatid mitochondria. The highly conserved ribosomal protein RPS12 is encoded on the maxicircle DNA (Feagin, 2000). A preliminary study in *Crithidia* identified two putative mitochondrial ribosomal proteins which are related to cytosolic ribosomal proteins S8 and S21 from a variety of organisms (Tittawella and Baranov, 2000). However, attempts to isolate mitochondrial ribosomes of trypanosomatids were only partially successful and their physical structure is essentially unknown (Shu and Göringer, 1998). None of the soluble factors (initiation and elongation factors) required for mitochondrial translation have been characterised with the exception of the tRNAs. Unlike in most other organism, all mitochondrial tRNAs are imported from the cytosol and therefore of the eukaryotic-type (see Section 4.3). This results in a paradoxical situation. Mitochondrial translation in trypanosomatids, representing one of the most derived prokaryotic-type translation systems known, somehow has to function exclusively with eukaryotic-type tRNAs imported from the cytosol (Fig. 1).

Many important questions concerning mitochondrial protein synthesis in trypanosomatids remain unanswered at present. Is the short length of the rRNAs compensated by a proportionally larger contribution of ribosomal proteins? How are the fully edited mRNAs selected for translation and partially edited ones excluded? How can such a derived ribosome function with eukaryotic-type tRNAs only?

4.3. tRNA import

Mitochondrial tRNA import occurs in many protozoa, in plants, some fungi and in a few invertebrates. However, no tRNA import has been detected in any vertebrate to date. Interestingly, the phylogenetic distribution of the process while widespread is disperse: amongst related organisms showing tRNA import, one often finds some species which do not show import and vice versa. Thus, mitochondrial tRNA import might have a polyphyletic origin (Schneider and Marechal-Drouard, 2000).

During evolution, most protein-encoding genes of the original endosymbiont were transferred to the nucleus, resulting in the situation that about 95% of mitochondrial proteins have to be imported into the mitochondria. The situation is different for tRNA import. There is no evidence for transfer of mitochondrial tRNA genes to the nucleus. Instead, the lack of mitochondrial tRNA genes is compensated for by import of a fraction (typically around 5%) of the corresponding nucleus-encoded tRNAs which are involved in cytosolic translation. A prediction from this scenario is that all imported tRNAs are of the eukaryotic-type. Therefore, no nucleus-encoded prokaryotic-type tRNAs are expected to exist. There is good experimental evidence that these predictions are accurate for all organisms known to import tRNAs (Schneider and Marechal-Drouard, 2000).

In most organisms which import tRNAs not all mitochondrial tRNA genes were lost. The situation in trypanosomatids is unusual since they have lost the entire set of their mitochondrial tRNA genes (Hancock and Hajduk, 1990; Simpson et al., 1989) (Fig. 1). The only other known taxonomic group where the same situation is found are the Apicomplexa (Feagin, 1992). Interestingly, the two groups are not closely related phylogenetically but they share a parasitic lifestyle. There are three main questions of interest concerning mitochondrial tRNA import in trypanosomatids. What features of a tRNA are recognised by the import system? What is the mechanism of tRNA import and which factors build the import machinery? Finally, what

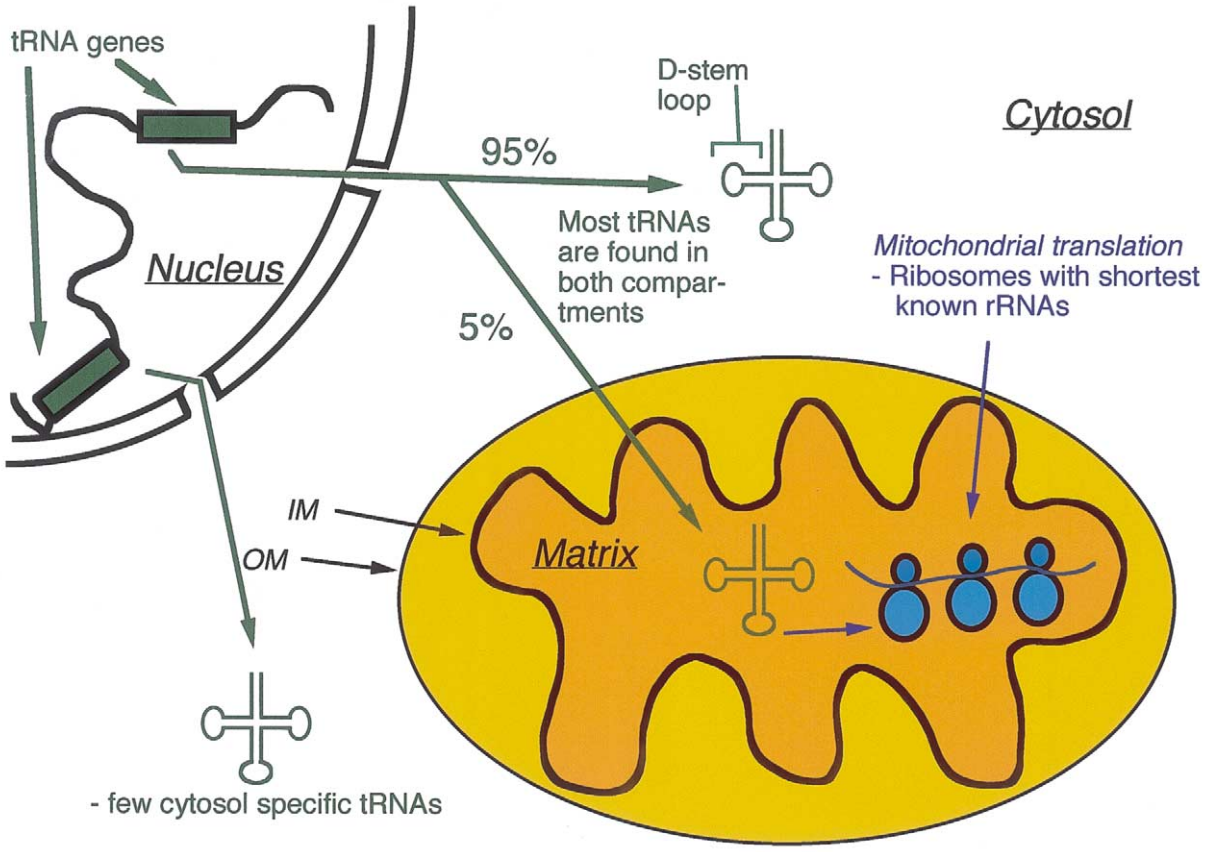

Fig. 1. Schematic overview of mitochondrial tRNA import (green) and organellar translation (blue) in trypanosomatids (disucussed in Sections 4.2 and 4.3). Most nucleus-encoded tRNAs are in part (ca. 5%) imported, whereas ca. 95% remain in the cytosol. Few cytosol-specific tRNAs exist. Mitochondrion-encoded tRNAs are absent. Mitochondrial translation therefore works with eukaryotic-type tRNAs only. Mitochondrial ribosomes of trypanosomatids have the shortest known rRNAs. OM, outer membrane; IM, inner membrane. The D-stem loop region of the tRNA, containing a putative mitochondrial targeting signal is indicated.

consequences do imported eukaryotic-type tRNAs have on the mitochondrial translation?

Except for a few cytosol-specific ones, all trypanosomal tRNAs can be found in both compartments. Even though there is not much specificity concerning the types of tRNAs which are imported, it is clear that other small RNAs are excluded from import. In vivo and in vitro studies suggest that in *Leishmania* the import substrates correspond to mature tRNAs (Adhya et al., 1997; Aphasizhev et al., 1998; Kapushoc et al., 2000; Rubio et al., 2000). A variant of the one leishmanial cytosol-specific tRNA known, which carried the D-stem loop of an imported one, was recovered in mitochondria when expressed in vivo (Lima and Simpson, 1996). The role of the D-stem loop (Fig. 1) as an import determinant was further supported by in vitro import experiments (Mahapatra et al., 1998; Rubio et al., 2000). In *T. brucei* the situation is more complex. An in vivo study suggested that tRNAs are imported into mitochondria independently of the genomic context as well as of their genetic origin (Hauser and Schneider, 1995). Even cytosolic tRNAs from yeast and humans could be imported when expressed in vivo. On the other hand, it has been suggested that the actual import substrates are 5′-extended tRNA precursors. A transcript consisting of the $tRNA^{Ser}$ and $tRNA^{Leu}$ separated by a 59 nucleotide long spacer has been detected in *T. brucei* (LeBlanc et al., 1999). Furthermore, this precursor was shown to be imported in an in vitro system, whereas the mature derivative was not (Yermovsky-Kammerer and Hajduk, 1999). However, precursor tRNAs were also found in *L. tarentolae*, where it is clear that mature tRNAs are the import substrates (Kapushoc et al., 2000). In agreement with this, the leishmanial precursor tRNAs were shown to be confined to the nucleus. Recently, characterisation of a mitochondrial fraction from *T. brucei* enriched for an RNase P-like activity was reported (Salavati et al., 2001). The enzyme was able to process in vitro transcribed tRNA precursors providing an argument for their involvement in mitochondrial import. More definitive evidence, however, requires the identification of the in vivo substrates of the enzyme.

A different but not less interesting aspect of mitochondrial tRNA import concerns its mechanism. In *S. cerevisiae*, the single imported tRNA is imported in complex with a mitochondrial precursor protein across the protein translo-

cation pore (Tarassov and Martin, 1996). In vitro tRNA import systems for *T. brucei* and two *Leishmania* species (Adhya et al., 1997; Rubio et al., 2000) show the following common features: pretreatment of mitochondria with proteinase abolished import, indicating the need for proteinaceous receptors on the surface of mitochondria. In *Leishmania tropica* it was shown that antibodies against a protease-sensitive RNA-binding protein of 15 kDa inhibited import (Adhya et al., 1997). The protein has, however, not been characterized any further. In all in vitro systems, import required external and probably internal ATP as well as one or both components of the electrochemical proton gradient (Mukherjee et al., 1999; Rubio et al., 2000; Yermovsky-Kammerer and Hajduk, 1999). Most importantly, none of the assays in *Leishmania* or *T. brucei* requires the addition of cytosolic factors, which argues that the import mechanism is different from yeast and therefore supports the polyphyletic origin of mitochondrial tRNA import.

Finally, imported eukaryotic-type tRNAs may not only create problems for the function of the highly derived mitochondrial ribosomes (see Section 4.2) but also interfere with other unique features of the prokaryotic-type translation system of mitochondria. One example concerns the variant genetic code, found in many mitochondria including the ones from trypanosomatids, where the stop codon UGA has been reassigned to tryptophan. The organellar $tRNA^{Trp}$ therefore has to decode UGA in addition to the normal tryptophan codon UGG. Suppression of UGA stop codons in the cytosol is expected to be harmful. In order to solve this problem, *L. tarentolae* imports the normal cytosolic $tRNA^{Trp}$, which is unable to decode the stop codon. Once inside the mitochondria, however, the CCA anticodon of the $tRNA^{Trp}$ gets converted to UCA by RNA editing allowing the tRNA to read both UGG and UGA codons (Alfonzo et al., 1999). Another problem concerns translation initiation. All organisms have initiator $tRNAs^{Met}$, but in prokaryotic-type systems only initiator $tRNA^{Met}$ carrying a formylated methionine functions in translation initiation. It should be mentioned, though, that in many mitochondria including the ones from trypanosomatids start codons other than AUG can be used. However, alternative start codons are exceptional within a mitochondrial genome. Most mitochondrial mRNAs are predicted to have an AUG start codon and therefore are expected to require a formylated initiator $tRNA^{Met}$. A $tRNA^{Met}$ formyl-transferase does not exist in the cytosol of eukaryotes and the structure of the eukaryotic inititator tRNA has distinct features which set it apart from its prokaryotic counterpart (RajBhandary, 1994). Translation initiation in trypanosomatid mitochondria, however, must function with eukaryotic-type tRNAs only. The mechanisms by which this is accomplished are not known. Finally, difficulties may be encountered with the mitochondrial $tRNA^{Gln}$. Glutaminyl-tRNA synthetase is generally absent in mitochondria and organellar $tRNAs^{Gln}$ are indirectly acylated in a two step process. They are first mischarged with glutamate by glutamyl-tRNA synthetase and the glutamate is then converted to glutamine by $tRNA^{Gln}$-dependent transamidase (Ibba and Soll, 2000; Schön et al., 1988). Eukaryotic and organellar $tRNAs^{Gln}$ look therefore quite different, since the latter one has to be a substrate for the mitochondrial glutamyl-tRNA synthetase. It was shown that in trypanosomatids, unlike in all other organisms investigated so far, a mitochondrial glutaminyl-tRNA synthetase exists. Furthermore, it was shown in *L. tarentolae* that the enzyme is distinct from its cytosolic counterpart (Nabholz et al., 1997). The evolutionary origin of the mitochondrial activity is unknown since its gene has not yet been identified. As illustrated by the two examples, exploring the limits of adaptation of a prokaryotic-type translation system to eukaryotic components may help to reveal fundamental requirements of translation.

5. Replication of the mitochondrial genome

Mitochondrial tRNA import is essential for the translation of mitochondria-encoded proteins. However, translation is only possible if during cell division the kinetoplast network is correctly replicated and distributed to the daughter cell. In this section, a short summary is presented on this formidable problem (Fig. 2). Many extensive reviews have been published on the subject (Ryan et al., 1988; Shlomai, 1994; Shapiro and Englund, 1995; Morris et al., 2001). The process involves two distinct steps, the replication of the actual DNA sequence and formation of the kDNA network.

The modes of replication for maxi- and minicircles appear quite similar. Both types of molecules are replicated unidirectionally via theta-like intermediates. Replication of the leading strand starts at the universal minicircle sequence. This 12mer nucleotide sequence is conserved in minicircles of most trypanosomatid species and can be present in more than one copy in a single molecule. Interestingly, a practically identical sequence can be found at the origin of replication in the *T. brucei* maxicircle DNA suggesting that the same proteins might be involved in replication initiation for both groups of molecules (Shapiro and Englund, 1995). DNA synthesis on minicircles depends on short RNA primers synthesised be a mitochondrial DNA primase. The enzyme has been purified from *C. fasciculata*. It is 28 kDa in size and able to synthesise short RNA pieces in vitro using single stranded DNA as a template (Li and Englund, 1997). The DNA polymerase, which replicates the bulk of mitochondrial DNA is, in analogy to other mitochondrial systems, expected to be of the gamma-type. Surprisingly, no such enzyme has been found in trypanosomatid mitochondria. The only mitochondrial DNA polymerase characterised is structurally and functionally related to beta-type DNA polymerases in other eukaryotes (Torri and Englund, 1995). These enzymes are involved in DNA repair in the nucleus and show gap filling activity. Since the newly replicated minicircles also contain gaps the protein may therefore have the same function in trypanosomatid mito-

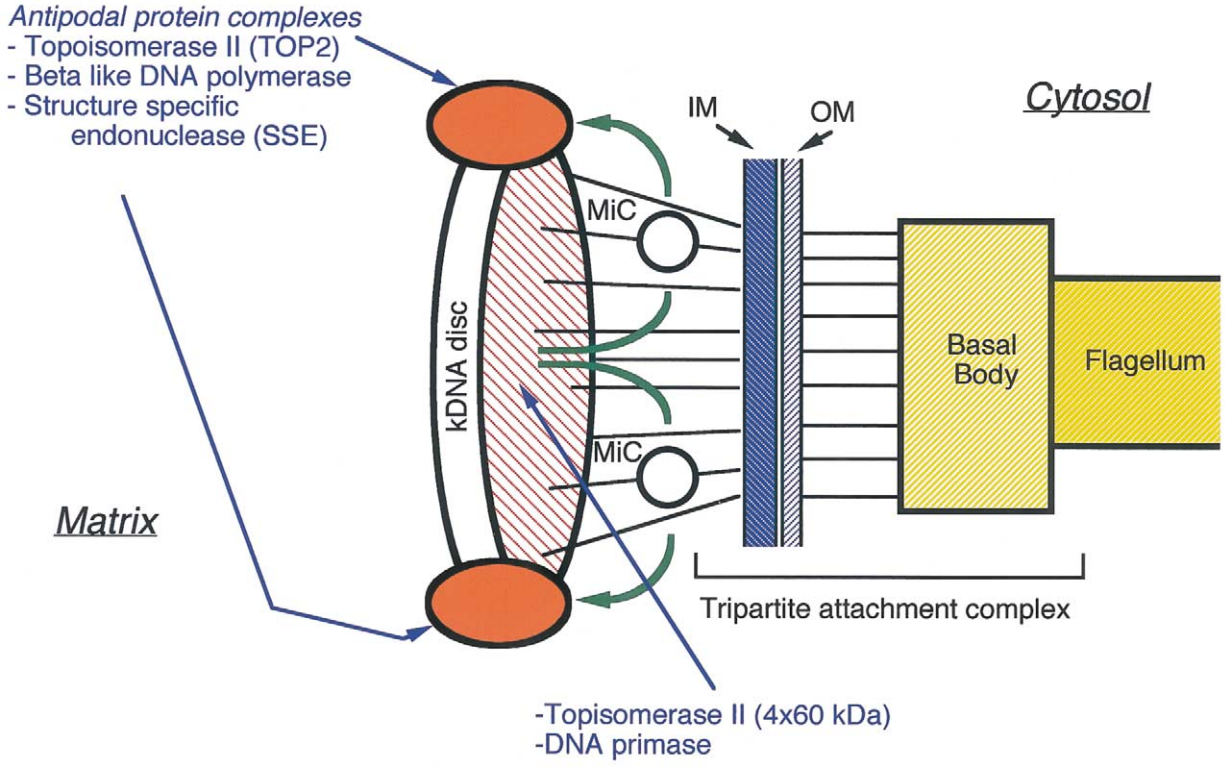

Fig. 2. Schematic view of a replicating kDNA network (discussed in Section 5). Minicircle (MiC) replicate as free monomers between the kDNA disc (green) and the membrane and reattach at two antipodal protein complexes at the edge of the network (red). Key enzymes involved in kDNA network formation are listed (blue) and their ultrastructural localisation is indicated. The DNA primase is localised at the two faces of the kDNA disc. The tripartite attachment complex links the kDNA to the basal body of the flagellum and plays an essential role in kDNA segregation.

chondria. Finally, a structure-specific endonuclease, showing RNase H activity which is probably required to remove the RNA primers has been isolated from *C. fasciculata* mitochondria. Sequence analysis of the 33 kDa protein revealed homology to the 5′-exonuclease domain of bacterial DNA polymerase I (Engel and Ray, 1998; Engel and Ray, 1999).

Not only the genetic information but also the topology of the kDNA has to be duplicated during cell division. The enzymes described above have to be spatially organised and timely regulated in a precise manner (Fig. 2). Maxicircles are replicated while integrated in the network. Minicircles, on the other hand, are released from the centre of the kDNA disc and replicated as free molecules. Interestingly, recent evidence in *C. fasciculata* suggests that minicircles release is vectorial and occurs only from the plane of the disc which faces the mitochondrial membranes and the basal body of the flagellum (Fig. 2; Drew and Englund, 2001). A topoisomerase II activity presumably responsible for this step has been identified in *C. fasciculata*. It consists of a tetramer of 60 kDa subunits and appears to use only covalently closed network-bound minicircles as a substrate. Submitochondrial localisation studies showed that the enzyme is found throughout the kDNA disc (Fig. 2; Shlomai, 1994). In the subsequent step, the newly synthesised minicircles containing nicks or gaps migrate to two opposite sites at the edge of the disc, where they are reattached to the network, before they become distributed over the entire periphery of the disc. In agreement with that scenario, the mitochondrial DNA primase involved in the initial stages of the replication is found uniformly at the top and the bottom of the kDNA disc which corresponds to the place where released minicircles are expected to be found (Li and Englund, 1997). The proteins involved in the later stages of the replication, such as the structure-specific endonuclease, beta-like DNA-polymerase and a distinct topoisomerase II (TOP2), consisting of two subunits of 132 kDa, are specifically localised to two antipodal complexes which coincide with the minicircle reattachment zone at the edge of the network (Melendy and Ray, 1987; Ferguson et al., 1992). Interestingly, there are two ways in which the reattached minicircles get laterally distributed. In *T. brucei*, they move bidirectionally out of the two protein complexes building two crescent shaped forms (termed ‘polar’ replication mode). In all other trypanosomatids, there appears to be a rotation of the kDNA disc relative to the two protein complexes leading to unidirectional distribution of the minicircles across the kDNA periphery starting from each of the protein complexes (termed ‘annular’ replication mode) (Guilbride and Englund, 1998). After completion of the kDNA replication, the network undergoes a dramatic but poorly understood remodelling. It increases in size and

finally splits in two, which again requires topoisomerase II activity.

In contrast to other eukaryotes where mitochondrial DNA replication occurs throughout the cell cycle the duplication of the kDNA is precisely regulated. It occurs during a discrete phase of the cell cycle, with timing close to the nuclear S phase (Ploubidou et al., 1999). Interestingly, the only other group of organisms known to replicate their mitochondrial DNA at a specific time in the cell cycle are the Apicomplexa (Preiser et al., 1996). The precise timing of kDNA replication is reflected by the dynamic localisation of some of the enzymes involved in the process. TOP2 and DNA polymerase beta are specifically localised at the two antipodal protein complexes during the kDNA replication, whereas at other times they behave differently (Johnson and Englund, 1998). The localisation of the primase, however, remains constant throughout the cell cycle. Not only the localisation of some key enzymes but also their expression might be regulated. TOP2 mRNA level peaks just prior to or at the peak of DNA synthesis in synchronised cultures (Mahmood and Ray, 1998). It therefore appears to be co-regulated with proteins involved in nuclear replication. The actual segregation of the newly formed kinetoplast network and the mitochondrion depends on a microtubule-mediated separation of the new and old flagellar basal body suggesting an essential function of the previously described physical linkage between kDNA and the basal body (tripartite attachment complex) (Fig. 2; Robinson and Gull, 1991; Gull, 1999).

Despite the fact that many questions concerning the kDNA network have been successfully addressed through experimental methods, the most fundamental one of why the trypanosomatid mitochondrial genome is organised in such a unique way remains unanswered (Borst, 1991).

6. Regulation of mitochondrial biogenesis during differentiation

Mitochondrial biogenesis is not only regulated during the cell cycle but in some trypanosomatid species also during the different stages of the life cycle. The best studied example is *T. brucei* (Priest and Hajduk, 1994a). The energy metabolism of the bloodstream form in the vertebrate host is different from that of the procyclic form in the midgut of the tsetse fly (Clayton and Michels, 1996; Tielens and VanHellemond, 1998; Turrens, 1999). In long slender bloodstream forms, glucose is metabolised to 3-phosphoglycerate in the glycosomes and net ATP-production occurs in the cytosol via substrate level phosphorylation associated with the conversion of 3-phospoglycerate to pyruvate. There is ample oxygen in the bloodstream and the cells respire by a mitochondrially localised alternative oxidase (TAO) (Chaudhuri et al., 1998). This enzyme receives electrons via ubiquinone from cytosolic glycerol-3-phosphate and transfers them to oxygen. There is no vectorial translocation of protons and no ATP is produced in the process. Oxidative phosphorylation does not occur in bloodstream forms, since the cytochromes and many Krebs cycle enzymes are absent. To complete the life cycle long slender forms need to differentiate into the stumpy forms, which are preadapted for life in the midgut of the fly (Hendriks et al., 2000). They express some enzymes of the citric acid cycle and appear to have NADH-dehydrogenase (complex I) of the respiratory chain. The transition to the procyclic form is characterised by a massive induction of mitochondrial biogenesis. The volume of the mitochondrion increases and a complete Krebs cycle as well as a fully functional respiratory chain are established. Procyclic cells produce ATP by two distinct pathways. One is oxidative phosphorylation using glutamate as the main respiratory substrate. Glutamate is derived from proline, the most abundant amino acid in the midgut of the fly (Clayton and Michels, 1996). All electrons appear to enter the respiratory chain at the level of the succinate dehydrogenase (Turrens, 1989; Allemann and Schneider, 2000). Furthermore, a NADH-dependent fumarate reductase activity has been described (Hernandez and Turrens, 1998). Besides, oxidative phosphorylation procyclic mitochondria can also produce ATP by substrate level phophorylation using a mitochondrial acetate:succinate CoA transferase/succinyl CoA synthetase cycle which produces acetate as an endproduct (vanHellemond et al., 1998). The largest and most complex component of the respiratory chain is the NADH-dehydrogenase, which in mammalian cells is composed of more than 43 subunits (Scheffler, 1999). The exact composition of the *T. brucei* enzyme is not known, though, at least six subunits of the NADH-dehydrogenase (ND1, 4, 5, 7–9) complex are known to be encoded by the mitochondrial genome and one nucleus-encoded subunit (NdhK) has been identified (Peterson et al., 1993). Even though many genes of NADH dehydrogenase have been identified, it is still controversial when during the life cycle NADH-dehydrogenase activity is actually expressed (Beattie and Howton, 1996; Turrens, 1989).

Both mitochondria-encoded as well as nucleus-encoded mitochondrial gene products are regulated during the life cycle (Fig. 3). Dyskinetoplastic mutants are viable as bloodstream forms. Mitochondrial translation therefore is not essential for survival in the mammalian host (Stuart, 1971) (however, see discussion of *Tb*REL1 in Section 4.1). In agreement with the fact that procyclic mitochondria contain 30-fold more steady-state rRNA than bloodstream cells, it appears that the abundance of the molecules is controlled at the level of RNA stability (Michelotti et al., 1992). Also the quantity of mRNAs for cytochrome *b* and cytochrome oxidase subunit II is increased during differentiation to the procyclic form (Priest and Hajduk, 1994a). Interestingly, the ND7 and ND8 transcripts behave differently and more mRNA is found in bloodstream cells. The regulation, however, occurs in all of these cases at the level of RNA editing. An upregulation of mitochondria-encoded gene products in procyclic trypanosomes is expected as they

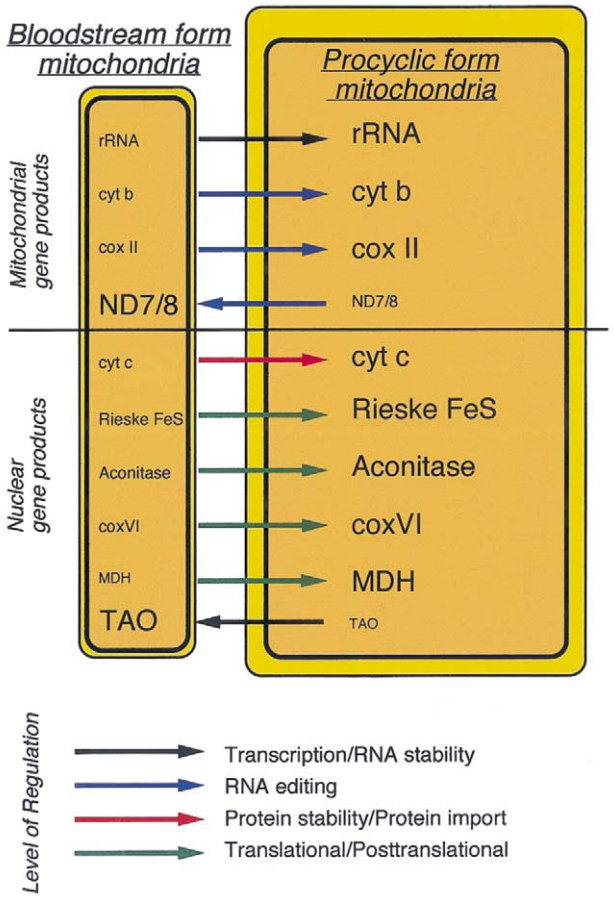

Fig. 3. Schematic view of the regulation of mitochondrial biogenesis during the life cycle of *T. brucei* (discussed in Section 6). Up- or downregulation is shown through the size of the letters and the direction of the arrows. The main mode of regulation is indicated by the colour of the arrow. Cyt *b/c*, cytochrome *b/c*; cox II/VI, cytochrome oxidase subunit II/VI; ND7, 8, subunits 7 and 8 of NADH-dehydrogenase; Rieske FeS, Rieske iron–sulfur protein; MDH, malate dehydrogenase; TAO, trypanosomal alternative oxidase.

are only used in that stage of the life cycle. However, why should the ND7 and ND8, subunits of NADH-dehydrogenase, be more abundant in bloodstream forms, considering the fact that mitochondrial translation is not essential? The answer is not known at present, but it has been suggested that NADH-dehydrogenase activity and therefore expression of ND7 and ND8 is required in short stumpy forms (Bienen et al., 1991). Elevated levels of ND7 and ND8 mRNA in long slender forms could therefore be a preadaptation to the differentiation into stumpy and later the procyclic forms.

There are many examples for stage-specific expression of nucleus-encoded mitochondrial proteins. The alternative oxidase, however, is the only case so far where the protein is more abundant (about 100-fold) in bloodstream than in procyclic forms. Interestingly, unlike in the examples described below, regulation appears to be exclusively through abundance of the transcript (Chaudhuri et al., 1998). Upregulation in procyclic mitochondria, on the other hand, is very common and has been described for cytochrome *c* (Torri and Hajduk, 1988), cytochrome *c*1, Rieske iron–sulfur protein (Priest and Hajduk, 1994b), aconitase (Saas et al., 2000), cytochrome oxidase subunit VI (Tasker et al., 2001) and mitochondrial malate dehydrogenase (Anderson et al., 1998) which are about 10–100-fold more abundant in procyclic cells. Whereas the expression of these proteins may be regulated at multiple levels it appears that the translational or even posttranslational modes of regulation play a major role. Stage-specific regulation has best been studied for cytochrome *c* which is 100-fold more abundant in procyclic cells than in bloodstream forms. The steady-state level of the corresponding mRNA in the two life cycle stages, however, differs by only by a factor of three to five. It was shown that the remaining large difference of the protein level (20-fold) is due to differential protein stability. The half-life of cytochrome *c* in bloodstream forms was shown to be about 1 h, whereas in procyclic cells the protein was essentially stable (Torri et al., 1993). What could be the mechanism of this differential stability? Cytochrome *c* is peripherally associated with the inner membrane facing the intermembrane space. Studies in *S. cerevisiae* have shown that cytochrome *c* which lacks the haem group can freely diffuse across the mitochondrial outer membrane before it gets modified in the intermembrane space by the attachment of a haem group. This step is catalysed by cytochrome *c* haem lyase and results in a structural change of cytochrome *c* which prevents it crossing the outer membrane (Kranz et al., 1998). Trypanosomes are haem auxotrophs and the concentration of haem is much higher in the midgut of the fly where digestive products of blood are found than in the vertebrate bloodstream where the haem is complexed to haemoglobin. Consequently, cytochrome *c* in bloodstream trypanosomes is expected to lack a haem group. Thus, it can presumably not be retained in the intermembrane space and may be rapidly degraded in the cytoplasm. Cytochrome *c* might therefore be the first example where stage-specific expression is regulated at the level of protein import (Priest and Hajduk, 1994a).

Even though import of none of the other trypanosomal proteins are known to be regulated in this manner, it is in principle an attractive method of controlling the abundance of mitochondrial proteins. Protein import has been characterised to some extent in procyclic cells using in vivo and in vitro import systems (Hauser et al., 1996; Priest and Hajduk, 1996). Overall, the process appears to be very similar to other eukaryotes. However, the amino-terminal presequences responsible for mitochondrial targetting appear to be exceptionally short (eight to nine amino acids) for many trypanosomatidal proteins (Clayton et al., 1995; Häusler et al., 1997). Recently, import of a constitutively expressed protein (NdhK) was reconstituted using a crude fraction of bloodstream form mitochondria. As in procyclic cells import was dependent on the membrane potential and matrix ATP (Bertrand and Hajduk, 2000). Comparing in vitro import of different substrate proteins into mitochondria

from procyclic and bloodstream forms will offer a novel approach to investigate stage-specific regulation.

7. Concluding remarks

Trypanosomatids have long been known to do things differently than other cells. Mitochondrial biogenesis is certainly no disappointment in that respect. The formation of their mitochondria requires completely unique biological processes such as gRNA-mediated RNA editing and the replication of the complex topology of the kDNA network. Furthermore, many of the more familiar processes show significant variations when compared with other organisms. Mitochondrial translation functions with a minimal contribution of rRNAs only and all mitochondrial tRNAs are of cytosolic origin. Differentiation during the life cycle offers the possibility to study the de novo formation of the respiratory complexes. This is all very exciting for the basic scientist. One should, however, not forget that many trypanosomatids are important medical pathogens of humans and livestock. The unique features of mitochondrial biogenesis in trypanosomatids therefore offer novel targets for chemotherapy of the diseases caused by these organisms. Whereas this may not exactly apply for *T. brucei* because many mitochondrial functions are dispensable in the vertebrate host, the situation is different for *Leishmania* and *T. cruzi* which rely on respiration throughout their entire life cycle.

Acknowledgements

I thank the members of my group and Adrian Streit for critical reviewing of the manuscript. This study was supported by grants 31-056825.99 and 4037-55154 from the Swiss National Foundation and by a fellowship of the 'Prof. Dr. Max Cloëtta'-Foundation.

References

Adhya, S., Ghosh, T., Das, A., Bera, S.K., Mahapatra, S., 1997. Role of an RNA-binding protein in import of tRNA into *Leishmania* mitochondria. J. Biol. Chem. 272, 21396–402.

Alfonzo, J.D., Blanc, V., Estevez, A.M., Rubio, M.A.T., Simpson, L., 1999. C to U editing of anticodon of imported mitochondrial $tRNA^{Trp}$ allows decoding of UGA stop codon in *Leishmania*. EMBO J. 18, 7056–62.

Allemann, N., Schneider, A., 2000. ATP production in isolated mitochondria of procyclic *Trypanosoma brucei*. Mol. Biochem. Parasitol. 111, 87–94.

Anderson, S.A., Carter, V., Parsons, M., 1998. *Trypanosoma brucei*: molecular cloning and stage-regulated expression of a malate dehydrogenase localized to the mitochondrion. Exp. Parasitol. 89, 63–70.

Aphasizhev, R., Karmarkar, U., Simpson, L., 1998. Are tRNAs imported into mitochondria of kinetoplastid protozoa as 5′-extended precursors? Mol. Biochem. Parasitol. 93, 73–80.

Attardi, G., Schatz, G., 1988. The biogenesis of mitochondria. Annu. Rev. Cell Biol. 4, 289–333.

Beattie, D.S., Howton, M.M., 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, 888–94.

Bertrand, K.I., Hajduk, S.L., 2000. Import of a constitutively expressed protein into mitochondria from procyclic and bloodstream forms of *Trypanosoma brucei*. Mol. Biochem. Parasitol. 106, 249–60.

Bienen, E.J., Saric, M., Pollakis, G., Grady, R.W., Clarkson, A.B., 1991. Mitochondrial development in *Trypanosoma brucei brucei* transitional bloodstream forms. Mol. Biochem. Parasitol. 45, 185–92.

Blum, B., Bakalara, N., Simpson, L., 1990. A model for RNA editing in kinetoplastid mitochondria: 'guide' RNA molecules from maxicircle DNA provide the edited information. Cell 60, 189–98.

Borst, P., 1991. Why kinetoplast DNA networks? Trends Genet. 7, 139–41.

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

Clayton, C., Häusler, T., Blattner, J., 1995. Protein trafficking in kinetoplastid protozoa. Microbiol. Rev. 59, 325–44.

Clayton, C.E., Michels, P., 1996. Metabolic compartmentation in African typanosomes. Parasitol. Today 12, 465–71.

delaCruz, V.F., Lake, J.A., Simpson, A.M., Simpson, L., 1985a. A minimal ribosomal RNA: sequence and secondary structure of the 9S kinetoplast ribosomal RNA from *Leishmania tarentolae*. Proc. Natl Acad. Sci. USA 82, 1401–5.

delaCruz, V.F., Simpson, A.M., Lake, J.A., Simpson, L., 1985b. Primary sequence and partial secondary structure of the 12S kinetoplast (mitochondrial) ribosomal RNA from *Leishmania tarentolae*: conservation of peptidyl-transferase structural elements. Nucleic Acids Res. 13, 2337–56.

Drew, M.E., Englund, P.T., 2001. Intramitochondrial location and dynamics of *Crithidia fasciculata* kinetoplast minicircle replication intermediates. J. Cell Biol. 153, 735–44.

Dyall, S., Johnson, P., 2000. Origins of hydrogenosomes and mitochondria: evolution and organelle biogenesis. Curr. Opin. Microbiol. 3, 404–11.

Engel, M.L., Ray, D.S., 1998. A structure-specific DNA endonuclease is enriched in kinetoplasts purified from *Crithidia fasciculata*. Nucleic Acids Res. 26, 4733–8.

Engel, M.L., Ray, D.S., 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, 8455–60.

Eperon, I.C., Janssen, J.W.G., Hoeijmakers, J.H.J., Borst, P., 1983. The major transcripts of the kinetoplast DNA of *Trypanosoma brucei* are very small ribosomal RNAs. Nucleic Acids Res. 11, 105–25.

Estevez, A.M., Simpson, L., 1999. Uridine insertion/deletion RNA editing in trypanosome mitochondria – a review. Gene 240, 247–60.

Feagin, J.E., 1992. The 6-kb element of *Plasmodium falciparum* encodes mitochondrial cytochrome genes. Mol. Biochem. Parasitol. 52, 145–8.

Feagin, J.E., 2000. Mitochondrial genome diversity in parasites. Int. J. Parasitol. 30, 371–90.

Feagin, J.E., Abraham, J.M., Stuart, K., 1988. Extensive editing of the cytochrome *c* oxidase III transcript in *Trypanosoma brucei*. Cell 53, 413–22.

Ferguson, M., Torri, A.F., Ward, D.C., Englund, P.T., 1992. In situ hybridization to the *Crithidia fasciculata* kinetoplast reveals two antipodal sites involved in kinetoplast DNA replication. Cell 70, 621–9.

Gillespie, D.E., Salazar, N.A., Rehkopf, D.H., Feagin, J.E., 1999. The fragmented mitochondrial ribosomal RNAs of *Plasmodium falciparum* have short A tails. Nucleic Acids Res. 27, 2416–22.

Gray, M.W., Burger, G., Lang, B.F., 1999. Mitochondrial evolution. Science 283, 1476–81.

Guilbride, D.L., Englund, P.T., 1998. The replication mechanism of kinetoplast DNA networks in several trypanosomatid species. J. Cell Sci. 111, 675–9.

Gull, K., 1999. The cytoskeleton of trypanosomatid parasites. Annu. Rev. Microbiol. 53, 629–55.

Hajduk, S.L., Harris, M.E., Pollard, V.W., 1993. RNA editing in kinetoplastid mitochondria. FASEB J. 7, 54–63.

Hancock, K., Hajduk, S.L., 1990. The mitochondrial tRNAs of *Trypanosoma brucei* are nuclear encoded. J. Biol. Chem. 265, 19208–15.

Hauser, R., Schneider, A., 1995. tRNAs are imported into mitochondria of *Trypanosoma brucei* independent of their genomic context and of their genetic origin. EMBO J. 14, 4212–20.

Hauser, R., Pypaert, M., Häusler, T., Horn, E.K., Schneider, A., 1996. In vitro import of proteins into mitochondria of *Trypanosoma brucei* and *Leishmania tarentolae*. J. Cell Sci. 109, 517–23.

Häusler, T., Stierhof, Y.-D., Blattner, J., Clayton, C., 1997. Conservation of mitochondrial targeting sequence function in mitochondrial and hydrogenosomal proteins from the early-branching eukaryotes *Crithidia*, *Trypanosoma* and *Trichomonas*. Eur. J. Cell Biol. 73, 240–51.

vanHellemond, J.J., Opperdoes, F.R., Tielens, A.G.M., 1998. Trypanosomatides produce acetate via a mitochondrial acetate: succinate CoA transferase. Proc. Natl Acad. Sci. USA 95, 3036–41.

Hendriks, E., vanDeursen, F.J., Wilson, J., Sarkar, M., Timms, M., Matthews, K.R., 2000. Life-cycle differentiation in *Trypanosoma brucei*: molecules and mutants. Biochem. Soc. Trans. 28, 531–6.

Hernandez, F.R., Turrens, J.F., 1998. Rotenone at high concentrations inhibits NADH-fumarate reductase and the mitochondrial respiratory chain of *Trypanosoma brucei* and *T. cruzi*. Mol. Biochem. Parasitol. 93, 135–7.

Horvath, A., Berry, E.A., Maslov, D.A., 2000a. Translation of the edited mRNA for cytochrome *b* in trypanosome mitochondria. Science 287, 1639–40.

Horvath, A., Kingan, T.G., Maslov, D.A., 2000b. Detection of the mitochondrially encoded cytochrome *c* oxidase subunit I in the trypanosomatid protozoan *Leishmania tarentolae*. J. Biol. Chem. 275, 17160–5.

Ibba, M., Soll, D., 2000. Aminoacyl-tRNA synthesis. Annu. Rev. Biochem. 69, 617–50.

Johnson, C.E., Englund, P.T., 1998. Changes in organization of *Crithidia fasciculata* kinetoplast DNA replication proteins during the cell cycle. J. Cell Biol. 143, 911–9.

Kable, M.L., Seiwert, S.D., Heidmann, S., Stuart, K., 1996. RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA. Science 273, 1189–95.

Kapushoc, S.T., Alfonzo, J.D., Rubio, M.A.T., Simpson, L., 2000. End processing precedes mitochondrial importation and editing of tRNAs in *Leishmania tarentolae*. J. Biol. Chem. 275, 37907–14.

Koslowsky, D.J., Bhat, G.J., Perrollaz, A.L., Feagin, J.E., Stuart, K., 1990. The MURF3 gene of *T. brucei* contains multiple domains of extensive editing and is homologous to a subunit of NADH dehydrogenase. Cell 62, 901–11.

Kranz, R., Lill, R., Goldman, B., Bonnard, G., Merchant, S., 1998. Molecular mechanisms of cytochrome *c* biogenesis: three distinct systems. Mol. Microbiol. 29, 383–96.

LeBlanc, A.J., Yermovsky-Kammerer, A.E., Hajduk, S.L., 1999. A nuclear encoded and mitochondrial imported dicistronic tRNA precursor in *Trypanosoma brucei*. J. Biol. Chem. 274, 21071–7.

Li, C., Englund, P.T., 1997. A mitochondrial DNA primase from the trypanosomatid *Crithidia fasciculata*. J. Biol. Chem. 272, 20787–92.

Lima, B.D., Simpson, L., 1996. Sequence-dependent in vivo import of tRNAs into the mitochondrion of *Leishmania tarentolae*. RNA 2, 429–40.

Madison-Antenucci, S., Sabatini, R.S., Pollard, V.W., Hajduk, S.L., 1998. Kinetoplastid RNA-editing-associated protein 1 (REAP-1): a novel editing complex protein with repetitive domains. EMBO J. 17, 6368–76.

Mahapatra, S., Ghosh, S., Bera, S.K., Ghosh, T., Das, A., Adhya, S., 1998. The D arm of tyrosine tRNA is necessary and sufficient for import into *Leishmania* mitochondria in vitro. Nucleic Acids Res. 26, 2037–41.

Mahmood, R., Ray, D.S., 1998. Nuclear extracts of *Crithidia fasciculata* contain a factor(s) that binds to the 5′-untranslated regions of TOP2 and RPA1 mRNAs containing sequences required for their cell cycle regulation. J. Biol. Chem. 273, 23729–34.

McManus, M.T., Shimamura, M., Grams, J., Hajduk, S.L., 2001. Identification of candidate mitochondrial RNA editing ligases from *Trypanosoma brucei*. RNA 7, 167–75.

Melendy, T., Ray, D.S., 1987. Purification and nuclear localization of a type I topoisomerase from *Crithidia fasciculata*. Mol. Biochem. Parasitol. 24, 215–25.

Michelotti, E.F., Harris, M.E., Adler, B., Torri, A.F., Hajduk, S.L., 1992. *Trypanosoma brucei* mitochondrial ribosomal RNA synthesis, processing and developmentally regulated expression. Mol. Biochem. Parasitol. 54, 31–41.

Missel, A., Souza, A.E., Nörskau, G., Göringer, H.U., 1997. Disruption of a gene encoding a novel mitochondrial DEAD-box protein in *Trypanosoma brucei* affects edited mRNAs. Mol. Cell. Biol. 17, 4895–903.

Morris, J.C., Drew, M.E., Klingbeil, M.M., Motyka, S.A., Saxowsky, T.T., Wang, Z., Englund, P.T., 2001. Replication of kinetoplast DNA: an update for the new millennium. Int. J. Parasitol. 31, 453–8.

Mukherjee, S., Bhattacharyya, S.N., Adhya, S., 1999. Stepwise transfer of tRNA through the double membrane of *Leishmania* mitochondria. J. Biol. Chem. 274, 31249–55.

Muller, U.F., Lambert, L., Goringer, H.U., 2001. Annealing of RNA editing substrates facilitated by guide RNA-binding protein gBP21. EMBO J. 20, 1394–404.

Nabholz, C.E., Hauser, R., Schneider, A., 1997. *Leishmania tarentolae* contains distinct cytosolic and mitochondrial glutaminyl-tRNA synthetase activities. Proc. Natl Acad. Sci. USA 94, 7903–8.

Nabholz, C.E., Speijer, D., Schneider, A., 1999. Chloramphenicol-sensitive mitochondrial translation in *Trypanosoma brucei*. Parasitol. Res. 85, 779–82.

Neupert, W., 1997. Protein import into mitochondria. Annu. Rev. Biochem. 66, 863–917.

Panigrahi, A.K., Gygi, S.P., Ernst, N.L., Igo, R.P., Palazzo, S.S., Schnaufer, A., Weston, D.S., Carmean, N., Salavati, R., Aebersold, R., et al., 2001. Association of two novel proteins, TbMP52 and TbMP48, with the *Trypanosoma brucei* RNA editing complex. Mol. Cell. Biol. 21, 380–9.

Peterson, G.C., Souza, A.E., Parsons, M., 1993. Characterization of a *Trypanosoma brucei* nuclear gene encoding a protein homologous to a subunit of bovine NADH: ubiquinone oxidoreductase (complex I). Mol. Biochem. Parasitol. 58, 63–70.

Ploubidou, A., Robinson, D.R., Docherty, R.C., Ogbadoyi, E.O., Gull, K., 1999. Evidence for novel cell cycle checkpoints in trypanosomes: kinetoplast segregation and cytokinesis in the absence of mitosis. J. Cell Sci. 112, 4641–50.

Preiser, P.R., Wilson, R.J., Moore, P.W., McCready, S., Hajibagheri, M.A., Blight, K.J., Strath, M., Williamson, D.H., 1996. Recombination associated with replication of malarial mitochondrial DNA. EMBO J. 15, 684–93.

Priest, J.W., Hajduk, S.L., 1994a. Developmental regulation of mitochondrial biogenesis in *Trypanosoma brucei*. J. Bioenerg. Biomembr. 26, 179–91.

Priest, J.W., Hajduk, S.L., 1994b. Developmental regulation of *Trypanosoma brucei* cytochrome *c* reductase during bloodstream to procyclic differentiation. Mol. Biochem. Parasitol. 65, 291–304.

Priest, J.W., Hajduk, S.L., 1996. In vitro import of the Rieske iron–sulfur protein by trypanosome mitochondria. J. Biol. Chem. 271, 20060–9.

RajBhandary, U.L., 1994. Initiator transfer RNAs. J. Bacteriol. 176, 547–52.

Rassow, J., Pfanner, N., 2000. The protein import machinery of the mitochondrial membranes. Traffic 6, 457–64.

Robinson, D.R., Gull, K., 1991. Basal body movements as a mechanism for mitochondrial genome segregation in the trypanosome cell cycle. Nature 352, 731–3.

Rubio, M.A., Liu, X., Yuzawa, H., Alfonzo, J.D., Simpson, L., 2000. Selective importation of RNA into isolated mitochondria from *Leishmania tarentolae* RNA. RNA 6, 988–1003.

Rusche, L.N., Huang, C.E., Piller, K.J., 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, 979–89.

Ryan, K.A., Shapiro, T.A., Rauch, C.A., Englund, P.T., 1988. Replication of kinetoplast DNA in trypanosomes. Annu. Rev. Microbiol. 42, 339–58.

Saas, J., Ziegelbauer, K., vonHaeseler, A., Fast, B., Boshart, M., 2000. A developmentally regulated aconitase related to iron-regulatory protein-1 is localized in the cytoplasm and in the mitochondrion of *Trypanosoma brucei*. J. Biol. Chem. 275, 2745–55.

Salavati, R., Panigrahi, A.K., Stuart, K.D., 2001. Mitochondrial ribonuclease P activity of *Trypanosoma brucei*. Mol. Biochem. Parasitol. 115, 109–17.

Scheffler, I.E., 1999. Mitochondria, Wiley-Liss, New York, NY.

Schnaufer, A., Panigrahi, A.K., Panicucci Jr, B., Salavati, R., Stuart, K., 2001. An RNA ligase essential for RNA editing and survival of the bloodstream form of *Trypanosoma brucei*. Science 291, 2159–62.

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, 234–41.

Schneider, A., Marechal-Drouard, L., 2000. Mitochondrial tRNA import: are there distinct mechanisms? Trends Cell Biol. 10, 509–13.

Schön, A., Kannangara, C.G., Gough, S., Söll, D., 1988. Protein biosynthesis in organelles requires misaminoacylation of tRNA. Nature 331, 187–90.

Seiwert, S.D., Stuart, K., 1994. RNA editing: transfer of genetic information of from gRNA to precursor mRNA in vitro. Science 266, 114–7.

Seiwert, S.D., Heidmann, S., Stuart, K., 1996. Direct visualization of uridylate deletion in vitro suggests a mechanism for kinetoplastid RNA editing. Cell 84, 831–41.

Shapiro, T.A., Englund, P.T., 1995. The structure and replication of kinetoplast DNA. Annu. Rev. Microbiol. 49, 117–43.

Shlomai, J., 1994. The assembly of kinetoplast DNA. Parasitol. Today 10, 341–6.

Shu, H.H., Göringer, H.U., 1998. *Trypanosoma brucei* mitochondrial ribonucleoprotein complexes which contain 12S and 9S ribosomal RNAs. Parasitology 116, 157–64.

Simpson, A.M., Suyama, Y., Dewes, H., Campbell, D.A., Simpson, L., 1989. Kinetoplastid mitochondria contain functional tRNAs which are encoded in nuclear DNA and also contain small minicircle and maxicircle transcripts of unknown function. Nucleic Acids Res. 17, 5427–45.

Simpson, L., 1972. The kinetoplast of hemoflagellates. Int. Rev. Cytol. 32, 139–207.

Sloof, P., Burg, J.V.d., 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, 4171–90.

Sogin, M.L., Elwood, H.J., Gunderson, J.H., 1986. Evolutionary diversity of eukaryotic small-subunit rRNA genes. Proc. Natl Acad. Sci. USA 83, 1383–7.

Sollner-Webb, B., 1996. Trypanosome RNA editing: resolved. Science 273, 1182–3.

Souza, A.E., Myler, P.J., 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, 2100–7.

Stuart, K.D., 1971. Evidence for the retention of kinetoplast DNA in an acriflavine-induced dyskinetoplastic strain of *Trypanosoma brucei* which replicates the altered central element of the kinetoplast. J. Cell Biol. 49, 189–95.

Stuart, K., Allen, T.E., Heidmann, S., Seiwert, S.D., 1997. RNA editing in kinetoplastid protozoa. Microbiol. Mol. Biol. Rev. 61, 105–20.

Tarassov, I., Martin, R., 1996. Mechanisms of tRNA import into yeast mitochondria: an overview. Biochimie 78, 502–10.

Tasker, M., Timms, M., Hendriks, E., Matthews, K., 2001. Cytochrome oxidase subunit VI of *Trypanosoma brucei* is imported without a cleaved presequence and is developmentally regulated at both RNA and protein levels. Mol. Microbiol. 39, 272–85.

Tielens, A.G.M., VanHellemond, J.J., 1998. Differences in energy metabolism between *Trypanosomatidae*. Parasitol. Today 14, 265–71.

Tittawella, I., 1998. Protein synthesis in mitochiondria isolated from the trypanosomatid protozoan *Crithidia fasciculata*. FEBS Lett. 423, 351–6.

Tittawella, I., Baranov, V., 2000. Localization of riboproteins in a trypanosomatid mitochondrion. FEBS Lett. 470, 319–24.

Torri, A.F., Bertrand, K.I., Hajduk, S.L., 1993. Protein stability regulates the expression of cytochrome *c* during the developmental cycle of *Trypanosoma brucei*. Mol. Biochem. Parasitol. 57, 305–16.

Torri, A.F., Englund, P.T., 1995. A DNA polymerase beta in the mitochondrion of the trypanosomatid *Crithidia fasciculata*. J. Biol. Chem. 270, 3495–7.

Torri, A.F., Hajduk, S.L., 1988. Posttranscriptional regulation of cytochrome *c* expression during the developmental cycle of *Trypanosoma brucei*. Mol. Cell. Biol. 8, 4625–33.

Turrens, J.F., 1989. The role of succinate in the respiratory chain of *Trypanosoma brucei* procyclic trypomastigotes. Biochem. J. 259, 363–8.

Turrens, J., 1999. More differences in energy metabolism between *Trypanosomatidae*. Parasitol. Today 15, 346–8.

Voelker, D.R., 1991. Organelle biogenesis and intracellular lipid transport in eukaryotes. Microbiol. Rev. 55, 543–60.

Yermovsky-Kammerer, A.E., Hajduk, S., 1999. In vitro import of a nuclearly encoded tRNA into the mitochondrion of *Trypanosoma brucei*. Mol. Cell. Biol. 19, 6253–9.