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Invited review

Natural and induced dyskinetoplastic trypanosomatids: how to live without mitochondrial DNA

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Abstract

Salivarian trypanosomes are the causative agents of several diseases of major social and economic impact. The most infamous parasites of this group are the African subspecies of the *Trypanosoma brucei* group, which cause sleeping sickness in humans and nagana in cattle. In terms of geographical distribution, however, *Trypanosoma equiperdum* and *Trypanosoma evansi* have been far more successful, causing disease in livestock in Africa, Asia, and South America. In these latter forms the mitochondrial DNA network, the kinetoplast, is altered or even completely lost. These natural dyskinetoplastic forms can be mimicked in bloodstream form *T. brucei* by inducing the loss of kinetoplast DNA (kDNA) with intercalating dyes. Dyskinetoplastic *T. brucei* are incapable of completing their usual developmental cycle in the insect vector, due to their inability to perform oxidative phosphorylation. Nevertheless, they are usually as virulent for their mammalian hosts as parasites with intact kDNA, thus questioning the therapeutic value of attempts to target mitochondrial gene expression with specific drugs. Recent experiments, however, have challenged this view. This review summarises the data available on dyskinetoplasty in trypanosomes and revisits the roles the mitochondrion and its genome play during the life cycle of *T. brucei*.

Keywords: *Trypanosoma*; Kinetoplast DNA; Dyskinetoplasty; Kinetoplast; RNA editing; Mitochondrial DNA

1. Introduction

The mitochondrial DNA (mtDNA) of trypanosomatid protozoa, also known as kinetoplast DNA (kDNA), is in many ways a remarkable structure. Historically, its characterisations by Bresslau and Scremin (1924), Meyer (1958), Steinert (1960) and others were among the first demonstrations that mitochondria harbour DNA. At the time, the kinetoplast was regarded as such an unusual structure that the general importance of these reports was not immediately recognised (Scheffler, 1999). Although the existence of mtDNA is now known to be a general feature of eukaryotic cells, its particular network structure in trypanosomatids is still exceptional (see below). The expression of the genes encoded in this DNA in many cases requires extensive RNA editing to form functional mRNAs. Finally, all the tRNAs required for translation of these mRNAs are imported from the cytosol and at least one of these tRNAs must be edited itself. The progress in the field of kDNA structure and expression in recent years is reflected by a number of reviews (Estevez and Simpson, 1999; Morris et al., 2001; Schneider, 2001; Stuart et al., 2002). However, for all its glory, kDNA is fragile. It is highly sensitive to drugs that intercalate into DNA or otherwise interfere with replication and kDNA is sometimes altered or even lost in nature, giving rise to induced and natural dyskinetoplastic (Dk) strains of trypanosomatids, respectively. This has led to the notion that kDNA is dispensable for certain stages of the life cycle or species of trypanosomatids. Recently, however, this view has been challenged (Schnaufer et al., 2001) and the aim of this review is to revisit the phenomenon of dyskinetoplasty and to summarise and, where necessary, reinterpret the conditions under which it can arise. In particular, we will address the following questions:

- What are the functions of the mitochondrion in the different life cycle stages of trypanosomes?
- Is mitochondrial gene expression and hence, mtDNA, required during the entire life cycle?
- If so, under what circumstances could these functions become non-essential for cell proliferation and allow dyskinetoplasty?

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2. Energy metabolism and mitochondrial biogenesis in trypanosomatids

2.1. Overview

Protozoan parasites of the family *Trypanosomatidae* are usually found in the body fluids of vertebrates and in the digestive tracts of insect or leech vectors (in the case of fish parasites). Some are parasites of plants. Since the vast majority of work on Dk trypanosomatids has been done on Salivarian trypanosomes (e.g. *Trypanosoma brucei* spp.) we will focus our discussion on parasites from this section of the genus. Here, the environmental switch from mammalian host (slender and stumpy bloodstream stages) to insect vector (procyclic, epimastigote, and metacyclic stages) and back is coupled to dramatic changes in the morphology and metabolism of the parasite (Hendriks et al., 2000). In particular, its single mitochondrion undergoes extensive remodelling at the structural as well as molecular level (Priest and Hajduk, 1994; Schneider, 2001). Whereas procyclic trypanosomes maintain a well developed mitochondrion with abundant cristae, slender bloodstream forms harbour a mitochondrial tube with scarcely any cristae. The latter lack cytochromes, several key enzymes of the Krebs cycle, and are incapable of oxidative phosphorylation. This remodelling reflects fundamental differences in the energy metabolism between the two life cycle stages (reviewed by Hill, 1976; Priest and Hajduk, 1994; Clayton and Michels, 1996; Tielens and Van Hellemond, 1998; Michels et al., 2000). Several characteristics of the trypanosome energy metabolism are still a matter of debate and some aspects of this controversy are important in understanding the significance of dyskinetoplasty. Furthermore, those Salivarian trypanosomes exhibiting natural kDNA alterations (*Trypanosoma equiperdum*, *Trypanosoma evansi*) have lost the capability to differentiate into the insect stage and are transmitted by biting flies or venereally (Brun et al., 1998). In this context the following summary attempts to highlight some major points.

2.2. The insect stage

In the procyclic stage, the parasite, which lives in the tsetse fly's midgut, produces most of its ATP through oxidative phosphorylation in the mitochondrion. The predominant sources for energy are amino acids such as proline, which are fed into the Krebs cycle. The presence of classic cytochrome-containing complexes (complexes III and IV) and of an ATP synthase complex (complex V) in the mitochondrial membrane is well established (Hill, 1976; Williams, 1994). The existence of a functional NADH:ubiquinone oxidoreductase (complex I) is a matter of debate (Tielens and Van Hellemond, 1998; Turrens, 1999) and it has been proposed that succinate functions as the primary electron donor (Turrens, 1989). Since both sides recently have presented further evidence for their favourite hypothesis (Christmas and Turrens, 2000; Fang et al., 2001) and since the presence or absence of a complex I, especially in the bloodstream stage, is of significance for the conditions under which dyskinetoplasty can arise, we will revisit this point below. *Trypanosoma brucei* also possesses a mitochondrial acetate:succinate CoA transferase (Van Hellemond et al., 1998) and can produce ATP through substrate level phosphorylation by converting acetyl-CoA to acetate. Depending on the trypanosome strain and the composition of the culture medium, the cytosolic/glycosomal substrate level phosphorylation via glycolysis is more or less active (ter Kuile and Opperdoes, 1992; Clayton and Michels, 1996). Glycolysis in kinetoplastids, to which *T. brucei* belongs, is unique in so far that its first seven steps are compartmentalised within the glycosome, a peroxisome-like organelle (Clayton and Michels, 1996; Michels et al., 2000; Parsons et al., 2001). In procyclics, most of the 1,3-biphosphoglycerate leaves the glycosome and is converted to phosphoenolpyruvate, which re-enters the glycosome. Here it is metabolised to malate. This step is important to maintain the ATP/ADP and redox balance within the glycosome. Finally, malate is shuttled out of the glycosome and oxidised to pyruvate, which is fed into the mitochondrial Krebs cycle (for a cartoon illustrating these pathways, see Tielens and Van Hellemond, 1998; Fig. 1).

2.3. The bloodstream stages

In the slender bloodstream stage, *T. brucei* relies exclusively on glycolysis and hence, substrate level phosphorylation for its ATP production. In contrast to the situation in procyclics, the bloodstream form mitochondrion has an important role in maintaining the glycosomal redox balance. Under aerobic conditions, glycerol 3-phosphate leaves the glycosome and is oxidised to dihydroxyacetone phosphate by a glycerol 3-phosphate oxidase, which is located in the intermembrane space (Allemann and Schneider, 2000). The glycosomal membrane is thought to be permeable for both glycerol 3-phosphate and dihydroxyacetone phosphate, thus facilitating this exchange (Clayton and Michels, 1996). The glycerol 3-phosphate oxidase is composed of an FAD-dependent glycerol 3-phosphate dehydrogenase and the trypanosome alternative oxidase. The enzyme uses oxygen as the electron acceptor and is sensitive to drugs such as salicyl hydroxamic acid and ascofuranone (Clarkson et al., 1989; Kornblatt et al., 1992; Minagawa et al., 1997; Chaudhuri et al., 1998). A role for complex I in transferring electrons to the glycerol 3-phosphate oxidase was suggested (Benne, 1990; Koslowsky et al., 1990; Beattie and Howton, 1996). Glycerol 3-phosphate oxidase is also expressed in procyclics, however, at greatly reduced levels (Opperdoes et al., 1981; Chaudhuri et al., 1995). Under anaerobic conditions, glycerol 3-phosphate is converted to glycerol, which diffuses out of the glycosome. This anaerobic pathway yields only half as much ATP per molecule of glucose (Clayton and Michels, 1996). Nevertheless, cells survive

hypoxic conditions and remain motile, although cellular ATP levels drop significantly (Opperdoes et al., 1976b). Similarly, inhibition of the glycerol 3-phosphate oxidase by salicyl hydroxamic acid or ascofuranone was reported to be lethal only under conditions where the anaerobic pathway was simultaneously inhibited by high concentrations of glycerol (Clarkson and Brohn, 1976; Brohn and Clarkson, 1978; Minagawa et al., 1997). Recent results, however, did show killing of *T. brucei* under anaerobic conditions or in the presence of low concentrations of salicyl hydroxamic acid (Helfert et al., 2001). Attempts by the same group to knockout the trypanosome alternative oxidase gene were unsuccessful. RNA interference (RNAi) studies could not definitely determine whether trypanosome alternative oxidase is essential to the bloodstream stage, but significant growth inhibition of the parasites was observed upon RNAi induction. These results raised the question whether glycerol 3-phosphate oxidase is an essential activity for slender bloodstream stages after all.

Clearly, the mitochondrion of the slender bloodform stage is anything but a 'sleeping' organelle. Besides its involvement in glycolysis it has been shown to be functional in transcription (Michelotti et al., 1992; Stuart and Feagin, 1992), polyadenylation (Bhat et al., 1992), RNA editing (reviewed in (Stuart et al., 1997)) and presumably also translation (Priest and Hajduk, 1994). The mitochondrion must also replicate its DNA, maintain its structural integrity and undergo division. In addition, the trypanosome mitochondrion, like those from other organisms, may harbour vital metabolic pathways (Scheffler, 1999) and roles in Ca^{2+} homeostasis, fatty acid metabolism, and apoptosis have been suggested (Linstead et al., 1977; Klein and Miller, 1981; Opperdoes et al., 1981; Vercesi et al., 1992; Wiemer et al., 1996; Welburn et al., 1997; Ridgley et al., 1999). Most, if not all, of these functions are performed by proteins that are encoded in the nuclear DNA and are imported into the mitochondrion. For this import process a membrane potential across the inner mitochondrial membrane ($\Delta\Psi$m) is absolutely required (Neupert, 1997; Bertrand and Hajduk, 2000). Slender bloodstream form *T. brucei* maintain a $\Delta\Psi$m, albeit of lower magnitude compared with procyclic forms (Nolan and Voorheis, 1992; Divo et al., 1993; Vassella et al., 1997; Bertrand and Hajduk, 2000). The mitochondrial F_0F_1-ATPase (complex V) was identified as the source for this membrane potential (Nolan and Voorheis, 1992, 2000).

This exclusive role of complex V in maintaining $\Delta\Psi$m was questioned for stumpy bloodstream forms where a participation of complex I was suggested (Bienen et al., 1991; Beattie and Howton, 1996). Stumpy bloodstream forms, which are non-proliferative, occur in pleomorphic cell lines when parasitaemia in the host increases (Vickerman, 1965). Monomorphic cell lines arise as the result of prolonged passage through mammalian hosts in the laboratory and, as the name suggests, have lost the ability to differentiate into stumpy forms and show decreased efficiency in the transformation into the procyclic form (Hendriks et al., 2000, and references therein). Stumpy bloodform stages were proposed to be necessary intermediates for the differentiation into the procyclic form (Hendriks et al., 2000, and references therein) and show increased mitochondrial metabolism. They maintain a higher $\Delta\Psi$m (Bienen et al., 1991; Divo et al., 1993; Vassella et al., 1997) and there is some evidence for ATP production in the mitochondrion (Bienen et al., 1993). Although cytochromes are still absent, some of the corresponding mRNAs can now be detected (in the case of nuclearly encoded subunits) or show RNA editing (in the case of mitochondrially encoded subunits) (Feagin et al., 1987; Feagin and Stuart, 1988; Hendriks et al., 2000) and tubular cristae begin to appear (Steiger, 1973).

3. Structure, replication, and expression of kDNA

3.1. Structure and replication of kDNA in T. brucei

Many features of the trypanosomal kDNA are very unusual, but the set of encoded genes is not (reviewed in Simpson, 1987; Stuart and Feagin, 1992; Shapiro and Englund, 1995; Feagin, 2000). In *T. brucei*, six subunits of complex I, apocytochrome b of CoQ-cytochrome c reductase (complex III), three subunits of cytochrome oxidase (complex IV), subunit A6 of ATP synthase (complex V), a ribosomal protein (RPS12), and two ribosomal RNA genes (9S and 12S) have been identified. Several of the identifications, however, are based on relatively weak homologies with other organisms. Five additional open reading frames so far have remained unidentified and are designated maxicircle unidentified reading frames (MURFs) 1, 2, and 5 and CR3 and 4 (GC-rich sequences). Nevertheless, it is apparent that the mitochondrial genome of *T. brucei*, like that of most other organisms, mainly, if not exclusively, contributes to the assembly of the respiratory chain and the mitochondrial ribosome (Scheffler, 1999). These genes are encoded in the so-called maxicircle component of the kDNA, making it the equivalent of mtDNA of other eukaryotes. Maxicircles in *T. brucei* are about 22 kb in size and range from 20 to 40 kb in other kinetoplastids. The second component of kDNA is the minicircle, measuring 1 kb in *T. brucei* and 0.9–2.5 kb in most other kinetoplastids. The function of these molecules was obscure until their role in RNA editing was discovered. They are now known to encode guide RNAs (gRNAs), small transcripts that provide the sequence information for the editing of mitochondrial mRNAs (see below and Estevez and Simpson, 1999; Stuart et al., 2001 or reviews on RNA editing). In *T. brucei*, most minicircles encode three gRNAs. Minicircle diversity is correlated with the extent of RNA editing and *T. brucei*, which shows the most extensive editing among kinetoplastids, contains 300–400 minicircle classes in its kDNA (Stuart and Feagin, 1992). About 40–50 maxicircles and

5000–10,000 minicircles are interlocked into a giant, disk-like network whose topology has been likened to the chain mail of medieval armour (Morris et al., 2001). The precise structure of this network and the mechanism of its replication have been the subject of several recent, authoritative reviews (Morris et al., 2001; Stuart and Feagin, 1992; Shapiro and Englund, 1995; Schneider, 2001).

Several proteins have been shown to be involved in kDNA replication. A topoisomerase II (topo II) is required for a number of steps. Minicircles are released from the centre of the disk and, after their replication, inserted at opposite sites at the edge of the disk. Both release and re-insertion require topo II activity. Although maxicircles are replicated within the network topo II is still required for this process. Finally, topo II activity is also thought to be involved in splitting the network in two after its replication is complete. A recent study confirmed the view that these different functions are carried out by more than one enzyme since knockdown of a particular topo II using RNAi primarily interfered with the re-attachment of minicircles (Wang and Englund, 2001). Because of its obvious importance for the parasite, topo II has been the focus of considerable attention as a drug target (Nenortas et al., 1998). Interestingly, several trypanocidal drugs such as berenil, pentamidine, ethidium bromide (EtBr), and isometamidinium chloride preferentially bind kDNA and generate minicircle–topo II complexes, leading to loss of kDNA and ultimately, Dk mutants (Shapiro and Englund, 1995). This is consistent with the fact that the above mentioned topo II knockdown also led to a Dk phenotype (Wang and Englund, 2001). The correlation of kDNA binding with trypanocidal activity is less clear and other targets have been suggested as well (Denise and Barrett, 2001). On the other hand, a Dk strain of *T. brucei* was shown to be resistant to berenil (Agbe and Yielding, 1995) and pentamidine resistance and dyskinetoplasty were found to be associated (Damper and Patton, 1976).

Besides topo II, a universal minicircle binding protein, a primase, DNA polymerase β, RNAse H, and several small basic proteins have been implicated in kDNA replication and some of these proteins are spatially organised with respect to the kDNA in a precise manner (reviewed in Morris et al., 2001; Schneider, 2001). The kinetoplast also appears to play a role in localisation of mitochondrial heat shock protein 70 (Klein et al., 1995). On a higher level of organisation, the kDNA network and the basal body outside of the mitochondrion are always in close proximity and are actually physically linked (Robinson and Gull, 1991). Segregation of kDNA network and mitochondrion are mediated by the basal body and the former occurs before mitosis is initiated. Although the extent of communication between kDNA/mitochondrion and the nucleus in trypanosomes is unclear, these events are highly co-ordinated and the existence of checkpoints has been suggested which would link the cell cycle to kDNA and/or basal body function or segregation (Das et al., 1994; Ploubidou et al., 1999).

3.2. Expression of mitochondrial genes

3.2.1. Overview

The expression of mitochondrial genes in trypanosomes requires four major processes: transcription of polycistronic precursor RNAs, transcript processing by cleavage/polyadenylation, editing (if required), and translation. There is clear evidence for the presence of the first three processes in both the bloodstream stages and the procyclic stage. Undisputed evidence for translation, however, so far exists for the procyclic stage only.

3.2.2. Transcription

A mitochondrial RNA polymerase from *T. brucei*, TBMTRNAP, has recently been cloned and characterised (Clement and Koslowsky, 2001). The gene for TBMTRNAP is transcribed in both the bloodstream and the procyclic stage (and, interestingly, also in a Dk strain), but nevertheless shows developmental regulation in that transcripts of different lengths are generated by alternative polyadenylation events. The actual production and activity of TBMTRNAP protein throughout the life cycle remains to be shown, since the existence of stable mRNAs in trypanosomes is not necessarily indicative of protein products (Priest and Hajduk, 1994). However, irrespective of the potential developmental regulation of TBMTRNAP activity, transcriptional activity in the mitochondrion throughout the life cycle is well established (Priest and Hajduk, 1994; Stuart et al., 1997). Some developmental control appears to take place on the level of transcript abundance. For instance, transcripts for the 9S and 12S rRNAs are 30-fold upregulated in the procyclic stage (Michelotti et al., 1992). Further regulation might occur via control of polyadenylation, resulting in varying lengths of poly(A) tails in certain transcripts (Bhat et al., 1992; Souza et al., 1992; Read et al., 1992, 1994a). Direct evidence for an influence of poly(A) tail length on the translational efficiency or the stability of mitochondrial transcripts has not yet been obtained.

3.2.3. RNA editing

A major role in regulating mitochondrial gene expression in trypanosomes has been suggested for RNA editing (reviewed in Stuart et al., 1997, 2002; Estevez and Simpson, 1999). Of the 18 ORFs encoded in the maxicircle of *T. brucei*, 12 are cryptogenes, i.e. their transcripts must undergo RNA editing to form functional mRNAs. This process inserts and, less frequently, removes uridine residues. RNA editing is very precise, although it can be quite extensive, leading to a doubling in transcript size. The – still hypothetical – regulatory role of RNA editing is based on the observation that completely edited transcripts from several genes have much greater abundance in certain stages of the life cycle. An overview is given in Table 1.

Transcripts encoding subunits of complex I are predominantly edited in the bloodform stage, whereas edited mRNAs for components of the cytochrome-containing

Table 1
Stage-specific RNA editing of mitochondrial transcripts

mRNA	Stage where editing peaks	Reference
ND3[a]	bf[b] and pf[c]	(Read et al., 1994b)
ND7, 5′ region	bf and pf	(Koslowsky et al., 1990)
ND7, 3′ region	bf	(Koslowsky et al., 1990)
ND8	bf	(Souza et al., 1992)
ND9	bf	(Souza et al., 1993)
CYb[d]	pf	(Feagin et al., 1987)
COXII[e]	pf	(Benne et al., 1986)
COXIII	bf and pf	(Feagin et al., 1988)
A6[f]	bf and pf	(Bhat et al., 1990)
RPS12[g]	bf	(Read et al., 1992)
MURF2[h]	bf and pf	(Feagin and Stuart, 1988)
CR3[i]	bf	(Stuart et al., 1997)
CR4	bf	(Corell et al., 1994)

[a] ND, NADH:ubiquinone oxidoreductase (complex I).
[b] bf, slender bloodstream form.
[c] pf, procyclic form.
[d] CYb, apocytochrome b (subunit of complex III).
[e] COX, cytochrome oxidase (complex IV).
[f] A6, ATP synthase subunit 6 (complex V).
[g] RPS12, ribosomal protein S12.
[h] MURF, maxicircle unidentified reading frame.
[i] CR, GC-rich sequence.

complexes III and IV generally peak in the procyclic stage. Some mRNAs, like the ones encoding subunit A6 of the ATP synthase complex and MURF2, show little or no stage-specific editing. The latter category also includes a few subunits of complexes I and IV. Interestingly, the mRNA for subunit ND7 contains two regions of extensive editing, only one of which is preferentially edited in the bloodstream stage. The mechanism of this regulation is unknown but does not reflect gRNA abundance (Koslowsky et al., 1992; Riley et al., 1994, 1995).

3.2.4. Translation

The stage-specific abundance of edited mRNAs reflects the metabolic differences between the life cycle stages to a certain degree. As discussed in Section 2, cytochrome-containing complexes III and IV are absent from slender bloodstream trypanosomes but are present in the procyclic insect stage (Priest and Hajduk, 1994). Recently, Horvath et al. (2000a,b) demonstrated that the mitochondrially encoded subunits of these complexes are actually translated within the mitochondrion of insect stage *Leishmania*. The pattern for edited ATP synthase subunit 6 mRNA is also consistent with the presence of the corresponding complex, which is expressed throughout the life cycle with a peak in the procyclic stage (Bienen and Shaw, 1991; Williams, 1994).

The situation is more complicated for complex I. As mentioned above, the significance and even existence of this complex in *T. brucei* is being debated (Tielens and Van Hellemond, 1998, 1999; Turrens, 1999). Several lines of evidence suggest the existence of a complex I in this organism. (I) Seven genes encoding subunits 1, 3, 4, 5, 7, 8, and 9 of this complex have been identified in the maxicircle DNA by sequence homology (Stuart and Feagin, 1992) and subunit K is encoded in the nuclear DNA (Peterson et al., 1993). (II) These genes are transcribed and, where necessary, edited, often in a stage-specific and concerted fashion. Specifically, transcription of subunits 4 and 5 (which do not require editing) and editing of subunits 7, 8, and 9 are upregulated in the bloodstream stage (Jasmer et al., 1985; Bhat et al., 1992; and references in Table 1). The other subunits are constitutively expressed (Peterson et al., 1993; and references in Table 1). (III) Several subunits have been demonstrated by immunoblotting of mitochondrial samples from bloodstream and procyclic stages and a putative complex I has been partially purified from procyclic mitochondria (Beattie and Howton, 1996; Fang et al., 2001). (IV) Respiration in crude mitochondrial fractions from both life cycle stages was found to be partially rotenone-sensitive (Beattie et al., 1994; Beattie and Howton, 1996; Fang et al., 2001). (V) The mitochondrial membrane potential was found to be sensitive to rotenone in the stumpy bloodform stage, and thus attributed to complex I activity (Bienen et al., 1991).

Several arguments have been made against a significant contribution, if any, of complex I to energy metabolism in trypanosomes. (I) NADH-dependent substrates were not able to stimulate ATP production in isolated mitochondria (Turrens, 1989; Allemann and Schneider, 2000). Instead, electrons were suggested to enter the respiratory chain at the level of succinate dehydrogenase and a separate NADH:-fumarate reductase was described as a potential generator of succinate in trypanosome mitochondria (Turrens, 1989; Christmas and Turrens, 2000). (II) The conclusions drawn from rotenone-sensitive inhibition were called into question due to comparatively high concentrations of inhibitor used in those experiments (Hernandez and Turrens, 1998; Turrens, 1999). (III) The identification of mitochondrial and nuclear genes as complex I subunits was questioned and it was suggested that these genes may represent other NADH-dependent proteins, including NADH:fumarate reductase (Turrens, 1999). Strain differences, differences in culture conditions, and the possibility of more than one NADH dehydrogenase activity have been suggested as possible explanations for these conflicting data and interpretations (Fang et al., 2001). In this context it is noteworthy that prolonged cultivation of insect stage *Leishmania tarentolae* cells led to a loss of gRNAs required for the editing of mRNAs encoding NADH dehydrogenase subunits (Thiemann et al., 1994; Gao et al., 2001). Complex I subunits also appear to be absent in cultured *Crithidia* cells (Speijer et al., 1997). This uncertainty about the function of the ND subunits encoded in the maxicircle DNA is unfortunate. The existence of a partially mitochondrially encoded complex with significance for the energy metabolism of bloodstream stages is of great interest for an understanding of dyskinetoplasty. Further studies, including knock-out experiments with respect to the nuclearly encoded subunit K, are clearly needed.

Apart from the putative assembly of subunit A6 into the ATP synthase complex and the debatable expression of

complex I in bloodstream stage *T. brucei*, direct evidence for the existence of mitochondrial translation in this stage of the life cycle does not exist. In contrast, mitochondrial translation in insect stage trypanosomatids has been demonstrated by numerous genetic (Schnaufer et al., 2000) and biochemical studies (Shaw et al., 1989; Speijer et al., 1996; Breek et al., 1997; Shu and Goeringer, 1998; Tittawella, 1998; Nabholz et al., 1999; Horvath et al., 2000a,b).

4. Dk species and strains

4.1. Overview

Trypanosomatids with altered kDNA have been the subject of many studies (Hajduk, 1978; Borst and Hoeijmakers, 1979; references therein and Table 2). These are broadly termed Dk organisms, the most extreme case being that of the akinetoplastic trypanosomatids, from which kDNA is totally absent. Dk organisms occur naturally and have been induced in the laboratory. The latter forms were initially generated by chemical mutagenesis (see Hajduk, 1978, for early references), but recent advances in the genetic manipulation of *T. brucei* have enabled the generation of Dk mutants by the inactivation of specific genes, (e.g. Wang and Englund, 2001). Notably, as discussed below, these latter mutants so far do not include any viable examples.

4.2. Naturally occurring Dk strains

Naturally occurring Dk cells were observed decades ago by the absence of the Giemsa-stainable kinetoplast from

Table 2
Representative dyskinetoplasty studies in chronological order

Organism (life cycle stage[a,b])	Conditions for dyskinetoplasty	Extent of dyskinetoplasty[c] and comments	Reference
T. evansi (bf)	Natural	100%	(Hoare and Bennett, 1937)
T. equiperdum (bf)	Natural (induced by rat passage)	100%	(Tobie, 1951)
		Show dispersed mtDNA is not kDNA	(Riou and Saucier, 1979)
Crithidia fasciculata (pf)	Acriflavine	85% by electron microscopy (Dk defined as aberrant kinetoplast)	(Hill and Anderson, 1969)
T. brucei (bf)	Acriflavine (mice)	100% by Giemsa	(Stuart, 1971)
T. equiperdum (bf)	Natural	100% by Giemsa, dispersed DAPI[d]-stained mtDNA	(Hajduk, 1976)
(1) *T. equiperdum* (bf)	Natural	(1) and (2), 99% by Giemsa, dispersed	(Hajduk, 1979)
(2) *T. equiperdum* (bf)	Acriflavine (mice)	DAPI-stained mtDNA	
(3) *C. fasciculata* (pf)	Acriflavine (culture)	(3) 50–80% by Giemsa	
T. equiperdum (bf)	BE[e] (rat)	100% by ultra-centrifugation, restriction analysis, hybridisation	(Riou and Benard, 1980)
T. equiperdum (bf)	1. EtBr[f] (rat), Acriflavine (rat)	100% by ultra-centrifugation, hybridisation	(Riou et al., 1980b)
T. brucei (bf)	Acriflavine (mice)	Clone 164/D3 reduced kDNA, no kinetoplast structure	(Stuart and Gelvin, 1980)
		Clone 164/D2 no kDNA	
		Detected by DNA hybridisation studies	
		Clone 164/D2 in vivo resistance to DNA-binding drugs and mislocalisation of hsp70	(Agbe and Yielding, 1995; Klein et al., 1995)
T. equinum (bf)	Natural	100% Giemsa	(Cuthbertson, 1981)
		Dispersed DAPI-stained mtDNA	
T. evansi (bf)	14 Month axenic culture	100% in 14 month cultures by DAPI	(Zweygarth et al., 1990)
T. brucei (bf)	(1) EtBr, (2) EtBr + VP[g], (3) BE, (4) BE + VP	(1) 44%, (2) 95%, (3) 73%, (4) 95%	(Agbe and Yielding, 1993)
		Detected by DAPI and EtBr after 72 h	
T. evansi (bf)	Para-rosaniline	100% by DAPI and Southern	(Silva-Tahat et al., 1995)
(1) *T. evansi* (bf)	6 Month axenic culture + clonal mice infection	(1) 65% after culture. mouse clones 100%	(Kaminsky et al., 1997)
(2) *T. equiperdum* (bf)		(2) 35% after culture. mouse clones 100%	
T. evansi (bf)	Natural	100% by DAPI, PCR	(Ventura et al., 2000)
T. brucei (pf)	RNAi of topo II	Gradual decrease in kDNA, by DAPI and hybridisation	(Wang and Englund, 2001)
C. fasciculata (pf)	EtBr	N/D[h]	(Biscardi et al., 2001)

[a] bf, bloodstream form.
[b] pf, procyclic (insect) form.
[c] Percentage of cells which were defined as dyskinetoplastic, i.e. normally no visible kinetoplast.
[d] DAPI, 4′, 6-diamidino-2-phenylindole stain.
[e] BE, berenil.
[f] EtBr, ethidium bromide.
[g] VP, verapamil.
[h] N/D, not determined.

insect and bloodstream stage trypanosomatids (see Hajduk, 1978). The percentage of spontaneously occurring Dk parasites was generally low and they were non-viable (Cosgrove and McSwain, 1960; Mühlpfordt, 1963a,b). Viable and established Dk parasites were only found among trypanosomatids, such as *T. equiperdum* ATCC 30023 (Tobie, 1951) and *T. evansi*[2] (Hoare and Bennett, 1937), which do not have an insect life cycle stage and thus have also been designated 'insect minus' (Opperdoes et al., 1976a). Both species have switched to other means of transmission, broadening their range of distribution beyond the tsetse belt of Africa (Brun et al., 1998). *Trypanosoma equiperdum* is transmitted venereally and infects equines. *Trypanosoma evansi* is transmitted by biting flies and, less frequently, bats, and infects a wide variety of mammals. The kDNA that was retained in some Dk strains was no longer organised into networks, but was shown by 4′,6-diamidino-2-phenylindole (DAPI) staining and electron microscopy to be dispersed in clumps throughout the whole mitochondrion (Hajduk, 1976, 1979; Vickerman and Tetley, 1977; Cuthbertson, 1981). A satellite DNA that is similar in buoyant density and amount to kDNA has been observed (Gutteridge et al., 1971; Renger and Wolstenholme, 1971; Hajduk and Cosgrove, 1979; Riou and Saucier, 1979) but appears to be unrelated to kDNA as discussed below (Riou and Pautrizel, 1977; for reviews, see Hajduk, 1978; Borst and Hoeijmakers, 1979; Englund, 1981).

Specific kDNA alterations on the molecular level have since been characterised in a variety of trypanosomatids, but especially in Salivarian trypanosomes. It was realised that all strains of *T. equiperdum* and *T. evansi*, whether they have a Giemsa-stainable kinetoplast or not, have lost functional parts of their mitochondrial genome and are thus Dk. *Trypanosoma equiperdum* strains have only a single, or at least very predominant, sequence class of minicircle DNA (Riou and Saucier, 1979; Frasch et al., 1980; Barrois et al., 1981; Silver et al., 1986) unlike *T. brucei*, which contains hundreds of minicircle sequence classes (Stuart and Feagin, 1992; and Section 3). The Pasteur strain of *T. equiperdum* contains 24.5 kb maxicircles that are apparently complete (Riou and Saucier, 1979) while the ATCC30019 strain of *T. equiperdum* has 14 kb maxicircles from which 13 genes have been lost and multiple DNA rearrangements have occurred (Frasch et al., 1980; Shu and Stuart, 1994). The single minicircle class is different between these strains of *T. equiperdum* (Barrois et al., 1981). *Trypanosoma evansi* strains lack maxicircle DNA and many have a single minicircle sequence class (Borst et al., 1987; Songa et al., 1990). Remarkably, the minicircle sequence is very similar, if not identical, among strains of *T. evansi* isolated from different regions of the world (Songa et al., 1990; Ou et al., 1991; Lun and Desser, 1995; and references therein). *Trypanosoma evansi* strains that are devoid of both maxi- and minicircle DNA (i.e. akinetoplastic) have been commonly reported (Riou and Saucier, 1979; Riou et al., 1980a; Ventura et al., 2000; and references therein). This is particularly true of strains from the New World where a study suggests that *T. evansi* strains devoid of kDNA are predominant in Brazil (Ventura et al., 2000).

The processes by which Dk forms arise in nature are unknown. The relatively high frequency of spontaneous Dk forms suggests that kDNA may be generally unstable. Nevertheless, many strains of *T. evansi* have retained at least one class of minicircle, in spite of its genetic irrelevance in the absence of a maxicircle. The homogeneity of minicircle populations in *T. evansi* and *T. equiperdum*, where they are present, may result from a combination of recombination among minicircles (Englund, 1981), their random segregation following replication (Morris et al., 2001), and a bias toward the most abundant minicircle type (Stuart and Gelvin, 1980). The presence of one type of minicircle in multiple *T. evansi* strains, together with results from isoenzyme analyses, suggests that they all arose from a single event that led to the loss of maxicircle DNA from *T. equiperdum* (Brun et al., 1998). Alternatively, selective pressure may have driven the changes in populations of minicircles. Treatment in vitro with arsenite, tunicamycin, or pentamidine resulted in dramatic and reversible changes in both the minicircle population and the maxicircle sequence, termed transkinetoplastidy, in *Leishmania* (Lee et al., 1992, 1993, 1994; Chiang et al., 1996; Basselin et al., 1998) and *T. cruzi* (Alves et al., 1994). In addition, minicircles that encode gRNAs for the editing of mRNAs for components of complex I are absent in the UC strain of *L. tarentolae*, leading to a subtle form of dyskinetoplasty (Thiemann et al., 1994; Gao et al., 2001). In this case, the loss of minicircles is probably due to selective culture conditions as a result of long-term laboratory maintenance. Similar events, leading to total loss of kDNA, have been observed in long-term axenic cultures of *T. equiperdum* and *T. evansi* (Zweygarth et al., 1990; Kaminsky et al., 1997).

4.3. Induced strains

Drugs that are used to treat and prevent trypanosomiasis are often DNA-binding drugs and can result in kDNA loss in trypanosomatids. Dyskinetoplasty was induced in bloodform *T. brucei* (Mühlpfordt, 1963a,b) and insect form *L. tarentolae* (Trager and Rudzinska, 1964) with concentrations of the drugs that resulted in their preferential accumulation in the mitochondrion. The drugs that induce dyskinetoplasty have been classified according to their mode of action. (I) DNA intercalators: acriflavine, EtBr, and methoxy-9-ellipticine and (II) non-intercalating drugs: hydroxystilbamidine, berenil, pentamidine, antrycide, and para-rosaniline (Hajduk, 1978).

Viable Dk strains of bloodstream *T. brucei* have been induced by repeated drug treatment during serial passage through laboratory rodents and monitoring by Giemsa stain-

[2] *T. equinum*, originally classified as a separate species, is now considered to be a form of *T. evansi* (Ventura et al., 2000, and references therein).

ing (Stuart, 1971; Riou and Benard, 1980; Agbe and Yielding, 1993). The resultant Dk strains initially retained mitochondrial DNA, as shown by DAPI staining and electron microscopy, despite the lack of a Giemsa-stainable kinetoplast (Stuart, 1971; Hajduk, 1976, 1979). Like naturally occurring Dk strains, induced strains were found to contain a satellite DNA that is similar in amount (~7–10%) and of the same buoyant density as kDNA (Stuart, 1971; Renger and Wolstenholme, 1971; see Englund, 1981, for additional references). However, restriction enzyme and hybridisation studies showed a relationship to nuclear DNA but not kDNA (Riou and Barrois, 1979; Riou and Saucier, 1979). Different clones from a Dk strain of *T. brucei* either retained some kDNA sequences or were devoid of kDNA (Stuart and Gelvin, 1980). Thus, both partial and total loss of kDNA can be chemically induced in bloodform trypanosomes by treatment with DNA-intercalating drugs (Riou and Benard, 1980; Riou et al., 1980b; Stuart and Gelvin, 1980). An electron-dense region was observed by electron microscopy in place of the kinetoplast, proximal to the flagellar pocket in drug-induced Dk *T. brucei* (Stuart, 1971; Hajduk, 1979) and in natural Dk *T. evansi* (Zweygarth et al., 1990; Ventura et al., 2000).

Total loss of kDNA has been induced in pleomorphic *T. brucei* strains (Stuart, 1971) and in *T. evansi* or *T. equiperdum* strains (Riou and Benard, 1980; Riou et al., 1980b; Silva-Tahat et al., 1995), which are generally monomorphic (Brun et al., 1998). Chemical induction of dyskinetoplasty in insect stage kinetoplastids (see Hill and Anderson, 1970; Hajduk, 1978 for early references), typically by addition of the drug to early log-phase cultures (e.g. Hajduk, 1979), resulted in non-viable Dk cells, presumably due to the requirement for maxicircle encoded gene products for energy metabolism in these cells (Vickerman and Preston, 1976). The growth and Dk production profiles of the insect forms suggest that they arise from cell divisions in which one daughter cell is Dk and non-viable, while the other one has apparently normal kDNA and is viable (Hajduk, 1979). Bloodstream stage trypanosomes undergo a large drop in cell number upon chemical induction, followed by appearance of a Dk population (Hajduk, 1979). These relapse profiles depend on the drug concentration (Stuart, 1971). It was suggested that non-viable daughter cells devoid of kDNA are generated, and that the eventual Dk strain arises from the cells with disrupted kDNA (Hajduk, 1979).

Specific interference with the activity of genes with roles in kDNA replication is now possible due to advances in genetic tools available for trypanosomatids. RNAi inactivation of topo II gene expression, which is associated with the kDNA replication machinery, led to inhibition of cell growth and gradual loss of kDNA in procyclic *T. brucei* (Wang and Englund, 2001). Surprisingly, as discussed below, this was also lethal to bloodstream forms (Wang Z. and Englund P.T., personal communication).

4.4. Common features

Overall, natural and induced Dk strains have been identified and can be classified as shown in Table 3. No cases where maxicircles are retained and minicircles are absent have been reported. This may reflect lack of detection due to the lower total amount of DNA represented by maxicircles compared to minicircles, or inevitable loss of maxicircles following loss of minicircles. The mitochondrion is always present, illustrating the fact that most mitochondrial components are encoded in nuclear genes. Both maxicircle and gRNA transcripts are produced if the corresponding DNA is retained, those transcripts are processed as in normal cells, and limited RNA editing occurs (Pollard and Hajduk, 1991; Shu and Stuart, 1994; Shu H.-H. and Stuart K., personal communication). The Dk *T. equiperdum* and *T. evansi* strains that lack maxicircles but contain minicircles produce corresponding gRNA transcripts (Gajendran et al., 1992).

The loss of kDNA sequences in Dk strains results in the loss of function of genes encoded in kDNA. Notably, all Dk *Trypanosoma* strains reported to date are able to survive as bloodstream forms but not as procyclic forms. Early evidence suggesting an essential role of the kinetoplast for cyclical development in the tsetse fly dates back to 1940, when Reichenow (1940) demonstrated that Dk *T. b. gambiense* were unable to survive in experimentally infected flies. It seems reasonable to assume that the survi-

Table 3
Alterations found in the kDNA of viable Dk trypanosomatids and functional consequences (for references, see text and Table 2)

Category	Ultrastructure (Giemsa)	Genome		Proteome	Example strains	
		Maxicircle	Minicircles		Natural	Induced
I	Normal	Normal	Less heterogeneous, or completely homogeneous	Loss of corresponding proteins	*T equiperdum* Pasteur	N/D[a]
II	Normal	Deletions	Less heterogeneous, or completely homogeneous	Loss of corresponding proteins	*L. tarentolae* UC strain *T. equiperdum* ATCC30019	N/D
III	Normal	Lost	Less heterogeneous, or completely homogeneous	Completely lost	*T. evansi* SH	N/D
IV	Akinetoplastic	Lost	Lost	Completely lost	Brazilian *T. evansi*	*T. brucei* 164/D2

[a] N/D, not determined.

val of Dk bloodstream forms reflects their reliance on glycolysis for energy production, unlike procyclic forms which rely on oxidative phosphorylation (Section 2). Clearly, energy generation by glycolysis does not absolutely require kDNA gene expression while oxidative phosphorylation does. However, while viable bloodstream form *Trypanosoma* that are devoid of kDNA can arise in nature or can be induced with drugs, gene-specific interference with kDNA replication or expression is lethal. Possible explanations for this conundrum are the subject of the next section.

5. Revisiting the function of mtDNA in the bloodstream stage

5.1. Bloodstream stages do not require mtDNA for survival. Or do they?

Even in those strains that retain detectable kDNA the absence of either the maxicircle (*T. evansi*) or all but one class of minicircles (*T. equiperdum*) must prevent the expression of functional proteins from the residual genome. This independence from mitochondrial gene products may represent a rare remodelling of metabolic pathways or simply the loss of a structure that, given alternative means of parasite transmission, is dispensable. The significance of this issue is illustrated by the question 'Does drug targeting of mitochondrial gene expression in bloodstream forms hold any promise?'. In order to give a satisfying answer to this question we need a more complete understanding of the contribution of the mitochondrial genes for proliferation and survival of the parasite. Treating trypanosomiasis by inhibiting mitochondrial processes such as replication or RNA editing is regarded with limited interest given the viability and virulence of Dk trypanosomes (Denise and Barrett, 2001). While various cellular functions are being considered as targets of trypanocidal drugs (Denise and Barrett, 2001), several of these agents inhibit replication of the mitochondrial genome (Nenortas et al., 1998). In addition, two aforementioned studies suggest that mitochondrial metabolism in bloodstream form *T. brucei* may be more complex than previously recognised. (I) Experimental knockdown in the slender bloodstream stage of an RNA ligase that is essential for RNA editing was lethal to the parasite (Schnaufer et al., 2001). Additional gene inactivation studies from our laboratory confirmed that RNA editing in general may be essential in this stage of the life cycle (Stuart et al., 2002). (II) Direct and rapid induction of dyskinetoplasty in bloodstream form *T. brucei* by gene inactivation (rather than by treatment with high doses of mutagenic drugs) was also lethal to the parasites (Wang Z. and Englund P.T., personal communication).

The time for Dk induction may be an important difference between the origin of viable and non-viable Dk strains. Both the natural and induced viable Dk strains appear to have occurred over many generations, providing an opportunity for step-wise selection. Notably, most studies indicating a vital function in bloodstream forms for mitochondrial gene expression or significant role for complex I used a monomorphic strain of 427 (Beattie and Howton, 1996; Schnaufer et al., 2001; Wang Z. and Englund P.T., personal communication). Strain differences are often suggested to account for confusing or conflicting results and it would be important to examine the effect of mitochondrial gene loss or inactivation in other, preferably pleomorphic strains.

What could be the cause of the lethality resulting from loss of kDNA or the expression of its genes? One possibility, applicable to both findings, is that the function of one or more proteins that are encoded in kDNA and subject to RNA editing, is normally essential in bloodstream forms. Alternatively, proteins of the RNA editing complex, all of which are encoded in nuclear DNA, may have a function in addition to RNA editing. Mitochondria from naturally occurring Dk *T. evansi* which retain minicircles and acriflavine-induced Dk *T. brucei* which are devoid of kDNA (Stuart and Gelvin, 1980) possess RNA editing complexes that catalyse editing in vitro, despite the irrelevance of this function in the absence of substrate RNA in vivo (Domingo et al., unpublished observation).

Below we identify kDNA genes that may have an essential role in the bloodstream stage and then discuss other possibilities involving more general cellular functions.

5.2. Potential functions of mitochondrial gene products in the bloodstream stage

5.2.1. Complex I

The possible role of respiratory complex I in the bloodstream stage of the life cycle is discussed above, in detail (Section 3.2). Inhibition of editing will almost certainly abolish complex I activity since several subunits of this complex are extensively edited. It has been suggested that complex I has a role in glycerol 3-phosphate oxidase activity (Benne, 1990; Koslowsky et al., 1990; Beattie and Howton, 1996), but glycerol 3-phosphate oxidase itself may not be essential since bloodstream form *T. brucei* can grow under anaerobic conditions (Opperdoes et al., 1976b). Nevertheless, recent experiments show that inhibition of glycerol 3-phosphate oxidase activity efficiently killed bloodstream form *T. brucei* in vitro and knocking down expression of its trypanosome alternative oxidase component significantly reduced their growth (Helfert et al., 2001). Whether complex I activity is associated with the glycerol 3-phosphate oxidase remains to be seen. Regulatable knockdown experiments involving the nuclearly encoded subunit K may shed some light on this question.

5.2.2. ATP synthase

The A6 subunit of the ATP synthase complex may also be essential in bloodstream forms. As discussed in Section 2.3, even slender bloodstream forms maintain a mitochondrial membrane potential difference, $\Delta\Psi$m, which is required for

protein import and thus is vital to the parasite. The mitochondrial F_0F_1-ATP synthase complex has been identified as the source of the $\Delta\Psi$m (Nolan and Voorheis, 1992, 2000). Subunit A6 is part of the F_0 component, is encoded in the maxicircle, and its mRNA requires extensive editing. The editing occurs in both bloodstream and insect stages of the life cycle but occurs preferentially in the bloodstream stage (Bhat et al., 1990), although ATP synthase is present at maximal levels in the procyclic stage (Williams, 1994). Since Dk strains preserve the structural integrity of the mitochondrion, which requires protein import, it follows that they also must maintain a $\Delta\Psi$m. Indeed, a Dk strain of *T. brucei* does possess a $\Delta\Psi$m and is functional in mitochondrial protein import (Klein et al., 1995). The simplest assumption is that the ATP synthase can function to maintain a $\Delta\Psi$m in the absence of subunit A6. In fact, in other cell types lacking the mitochondrial genome (rho^0 cells), namely mutants of the yeast *Saccharomyces cerevisiae* and of certain cultured human cells, incomplete ATP synthases lacking their mitochondrially encoded subunits do generate a membrane potential (Chen and Clark-Walker, 1999; Appleby et al., 1999). These incomplete ATPases are tethered more loosely to the membrane and retain their azide sensitivity but are oligomycin-insensitive (Appleby et al., 1999; Chen, 2000). This is consistent with the correlation of dyskinetoplasty and oligomycin-insensitive ATPase activity in *T. brucei* (Opperdoes et al., 1976a). Petite yeast strains with partial mtDNA deletions (rho^-) or those devoid of this DNA (rho^0) can grow on fermentable, but not on non-fermentable media. However, the α and β subunits of ATP synthase are essential for their growth, presumably because they are needed to maintain a $\Delta\Psi$m (Chen and Clark-Walker, 1999). The yeast *Kluyveromyces lactis* does not normally survive the loss of its mtDNA but mutants with mtDNA deletions have been isolated and found to have compensatory mutations in some of the nuclearly encoded ATP synthase subunits (Clark-Walker et al., 2000; Chen, 2000). These mutations apparently allow assembly of a partially functional complex, despite the absence of the mitochondrially encoded subunits. It is not known whether subunit A6 is required for a functional ATP synthase complex in *T. brucei*. Analogous to the situation in yeast, *T. brucei* strains may differ in this requirement and thus in their ability to survive loss of their kDNA. Alternatively, induction of Dk *T. brucei* may require selection for compensatory mutations as in *K. lactis*.

5.2.3. Other genes

Finally it should be pointed out that the function of five maxicircle reading frames remains elusive, three of which, MURF2, CR3, and CR4, give rise to transcripts that require extensive editing (Stuart and Feagin, 1992). Based on the hydrophobicity of the predicted gene products, CR3 and 4 were suggested to code for parts of respiratory complex I (Stuart et al., 1997), but it is possible that they serve a different function with significance for survival of bloodform stage parasites.

5.3. Other mitochondrial functions

5.3.1. Mitochondrial biogenesis is tied into the cell cycle

Mitochondrion division and cytokinesis are highly coordinated, as discussed above (Section 3.1) and replication of the kDNA network always occurs prior to mitosis. In addition, the kDNA network, while within the mitochondrion, has a close physical interaction with the flagellar basal body (Robinson and Gull, 1991). It has been suggested that checkpoints exist which link the cell cycle to kDNA and/or basal body function or segregation (Das et al., 1994; Ploubidou et al., 1999). Thus, the presence of kDNA may be required for normal cell division, either by signalling a successful replication or for structural reasons. Such requirements, if they exist, must be escaped in Dk strains.

5.3.2. Programmed cell death

A more exotic explanation for the lethal effects described above would be that the events initiated by these manipulations do not lead to the loss of an essential function, but instead, trigger a series of events leading to programmed cell death. Programmed cell death in trypanosomatids was suggested to regulate parasitaemia and differentiation in the insect vector and the coat of procyclin molecules present on the surface of procyclic stages was shown to be involved (Welburn et al., 1997; Pearson et al., 2000). It is not clear if similar mechanisms are relevant for bloodstream forms. However, a gene up-regulated in experimentally induced apoptosis in the procyclic stage was also found to be up-regulated in terminally differentiated bloodstream forms (Welburn and Murphy, 1998). Mitochondria play an important role in the early events of programmed cell death and a fall in $\Delta\Psi$m, induced by so-called permeability transition and involving complex I, was observed as one of the first events in the apoptotic cascade, prior to fragmentation of nuclear DNA (Scheffler, 1999; Fontaine and Bernardi, 1999). Other common factors for programmed cell death involving mitochondria include reactive oxygen species and Ca^{2+} homeostasis and these factors were also shown to be associated with cell death in *T. brucei* (Ridgley et al., 1999).

It seems possible that experimental manipulation of the integrity or expression of kDNA mimics events that induce apoptotic pathways in the parasite. Given the high co-ordination of mitochondrial and cellular division in *T. brucei*, it seems possible that, as pointed out by Pearson and coworkers with respect to the procyclic stage, ‘aberrant cell cycle events leading to multinucleation and altered cytokinesis … are a prelude to death’ (Pearson et al., 2000).

6. Concluding remarks

Trypanosomatids which lack kDNA can survive as blood-

stream forms. The apparently conflicting observation that they cannot survive the inactivation of kDNA replication or gene expression can be resolved if these organisms possess mechanisms that can bypass the requirement for the kDNA. Such mechanisms of genetic and/or epigenetic nature may entail the selection for compensatory mutations or adaptations. The powerful array of tools for genetic manipulation of trypanosomes and global genome expression profiling at the RNA and protein levels allows testing of these hypotheses.

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