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1471-4922/$ – see front matter
doi:10.1016/j.pt.2008.06.005 Available online 7 August 2008

Research Focus

What happens when *Trypanosoma brucei* leaves Africa

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Julius Lukeš and co-workers evaluated the evolutionary origin of *Trypanosoma equiperdum* and *Trypanosoma evansi*, parasites that cause horse and camel diseases. Although similar to *T. brucei*, the sleeping-sickness parasite, these trypanosomes do not cycle through the tsetse fly and have been able to spread beyond Africa. Transmission occurs sexually, or via blood-sucking flies or vampire bats. They concluded that these parasites, which resemble yeast petite mutants, are *T. brucei* sub-species, which have evolved recently through changes in mitochondrial DNA.

Trypanosoma brucei and kinetoplast DNA

Trypanosoma brucei undergoes a complex life cycle within its mammalian host and tsetse-fly vector. Two life-cycle stages, the mammal-infective bloodstream form (BSF) and the procyclic form (PCF, which normally resides in the insect midgut), are easily cultured in the laboratory and are the subjects of this commentary. The single mitochondrion of *T. brucei* contains an amazing genome, kinetoplast DNA (kDNA), which is a network of interlocked DNA rings [1]. The rings include dozens of maxicircles and thousands of minicircles. Maxicircles encode ribosomal RNA (rRNA) and a handful of proteins, which are mostly subunits of respiratory complexes, but they cannot be translated until they are edited by the insertion or deletion of uridylates at specific internal sites [2,3]. Small guide RNA templates, which are mostly encoded by minicircles, determine editing specificity. Because most *T. brucei* maxicircle transcripts are edited, some extensively, many guide RNAs are needed and, therefore, the kDNA network carries a startling array of minicircle sequence classes. Because most minicircles encode essential guide RNAs, trypanosome viability depends upon preserving the minicircle repertoire. Mathematical modeling predicts that the random segregation of *T. brucei* minicircle progeny would lead to the rapid loss of essential minicircles, but this does not happen [4,5]. This is probably because the network structure and its complex replication mechanism have evolved to preserve the minicircle repertoire [6,7]. However, the replication mechanism must not provide precise segregation of minicircle progeny because there is considerable drift in minicircle copy number during two years of laboratory culture [5]. Another factor that contributes to the preservation of the minicircle repertoire is trypanosome mating, which occurs only in the tsetse vector and results in mixing of the parental minicircles [8]; thus, mating could rescue minicircles that are in danger of being lost [4].

Lai *et al.* [9] have extensively discussed the evolutionary origins of *Trypanosoma equiperdum* and *Trypanosoma evansi*. In this commentary, we do not address this controversial and complex subject (for an alternative view, see Ref. [10]). Instead, we focus on the molecular changes that have altered the biology of these trypanosomes and occurred during their departure from Africa.

T. equiperdum and T. evansi

It has been known for many years that *T. equiperdum* and *T. evansi* differ markedly from *T. brucei* in their kDNA structure, and Lai *et al.* [9] have extended these conclusions

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to additional strains. Both parasites have homogeneous minicircles. Although all *T. evansi* strains are completely deficient in maxicircles, *T. equiperdum* shows more variation, with some strains that carry apparently complete maxicircles (although they could have point mutations inactivating essential genes); others are missing one or a few genes; and a few, like *T. evansi,* lack the entire maxicircle [9].

T. equiperdum and *T. evansi* can propagate only as BSFs and cannot survive in the tsetse fly. There are some *T. brucei* BSF strains (but not PCFs) that can also survive with a partial (dyskinetoplastic, Dk) or complete (akinetoplastic, Ak) loss of kDNA. These kDNA-deficient *T. brucei* strains appear naturally or can be induced by drugs, such as ethidium bromide [11]. Because wild-type BSFs produce ATP via glycolysis, the expression of many of its mitochondrial functions, including electron transport, is inhibited. However, PCFs have a well-developed and fully functional mitochondrion. Thus, it was thought for years that PCFs have a clear need for maxicircle gene products but BSFs do not. Furthermore, the existence of Ak trypanosomes led to the widespread belief that kDNA was not required for BSFs. However, the surprising discovery that editing enzymes are required for viability of Ak BSFs [12] soon led to the realization that at least one maxicircle gene product is required for BSF proliferation [13,14]. To appreciate the biology of *T. equiperdum, T. evansi* and the kDNA-deficient strains of *T. brucei*, it is essential to understand some features of mitochondrial physiology that are described in the following section.

Mitochondrial function depends upon membrane potential

Although mitochondria are most famous for producing ATP (through oxidative phosphorylation), they have key roles in a wide range of other cellular and metabolic processes [15]. Thus, mitochondria are essential organelles for virtually all eukaryotic cells. Because the vast majority of mitochondrial enzymes are encoded on nuclear genes, they are imported from the cytosol and sorted to the mitochondrial outer membrane (OM), inner membrane (IM), intermembrane space (IMS) or matrix. Like oxidative phosphorylation, mitochondrial protein import depends upon a membrane potential ($\Delta\Psi$) across the IM [16,17]. Similarly, $\Delta\Psi$ drives the transport of metabolites and other small molecules [18]. In many cells, such as *T. brucei* PCFs, the proton-pumping electron transport chain is thought to generate $\Delta\Psi$ (Figure 1a).

When the electron transport machinery is unavailable, cells often use a different scheme to generate $\Delta\Psi$ – running the ATP synthase in reverse (Figure 1b). For example, *T. brucei* BSFs use the ATP synthase to hydrolyze ATP (produced by glycolysis), thus pumping protons from the matrix to the IMS to generate $\Delta\Psi$ [13,14,19,20]. BSF trypanosomes are, therefore, dependent on the ATP synthase but not the respiratory chain. But there is a problem – because the synthase A6 subunit is encoded by maxicircles (and, possibly, other ATP-synthase subunits are encoded by maxicircle unidentified reading frames, or MURFs), how do Ak cells survive?

The mitochondrial membrane potential in kDNA-deficient BSF trypanosomes

Because trypanosomes lacking kDNA do not produce A6, neither of the two methods described in the previous paragraph can be used to generate $\Delta\Psi$. Instead, such trypanosomes use a third scheme, which was first uncovered by the analysis of petite yeast mutants, to generate $\Delta\Psi$ [21]. Yeast cells without mitochondrial DNA (which are known as 'petite' or 'rho°') lack a functional electron transport chain and are also missing the proton-pumping membrane component (F_o) of the ATP synthase. The loss of F_o in petite cells releases a soluble, catalytically active domain of the synthase (F_1) into the matrix. ATP hydrolysis by F_1 produces ADP, and the subsequent exchange of the ADP^{-3} for cytosolic ATP^{-4}, via the inner-membrane ADP–ATP carrier,

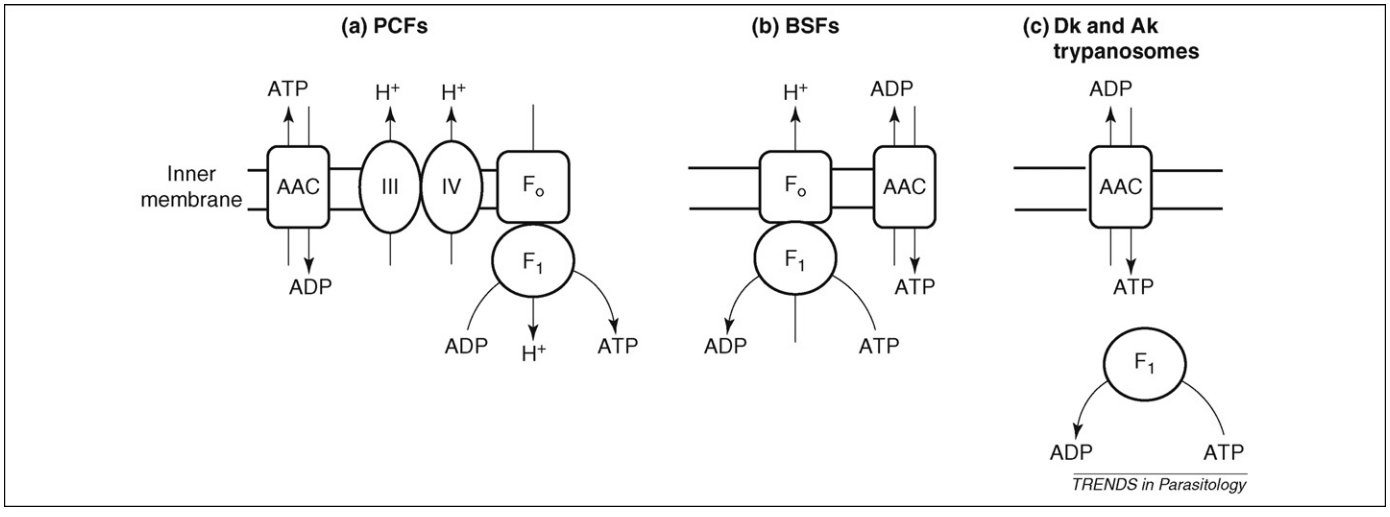

Figure 1. Mitochondrial inner-membrane potential in trypanosomes. The ATP synthase is composed of F_o, which is embedded in the inner membrane (IM) and translocates protons, and F_1, which can either synthesize or hydrolyze ATP. The ATP–ADP carrier (AAC) mediates the exchange of ATP and ADP across the IM. **(a)** In the PCF, the electron transport machinery (only complex III and IV are shown) generates $\Delta\Psi$, which is used to drive ATP synthesis, in addition to protein import and metabolite transport (not shown). **(b)** In the BSF, the ATP synthase runs backwards and uses ATP hydrolysis to pump protons across the IM to generate $\Delta\Psi$. **(c)** In Dk or Ak trypanosomes, the F_o portion of the ATP synthase is missing (e.g. owing to a lack of the kDNA-encoded subunit, A6), but the F_1 portion hydrolyzes ATP to ADP in the matrix. The exchange of ADP^{-3} with ATP^{-4} from the cytosol establishes $\Delta\Psi$.

Box 1. Possible steps in kDNA loss

Step 1. A trypanosome undergoes a spontaneous mutation in a maxicircle. This could be a point mutation in an essential gene or the deletion of one or more genes, but the A6 subunit of the ATP synthase (plus any MURF-encoded subunit of F_o) must remain functional. There is experimental evidence that the stochastic segregation of maxicircles could cause fixation of this mutation in all of the maxicircles of a cell within a short time (i.e. several months) [25]. This mutant trypanosome can no longer passage through the tsetse fly and is restricted to the bloodstream. These events could occur before or after an infected animal moves from the tsetse area as long as it can be transmitted non-cyclically. Once it has fully adapted to a non-tsetse-mediated transmission, it can leave Africa.

Step 2. With the cells propagating only in the bloodstream, there is no selection for minicircles encoding guide RNAs (gRNAs) except for those used for editing of F_o subunits (e.g. A6 and possibly a MURF). Given the imprecision of minicircle segregation enabled by its replication mechanism (see main text), minicircle homogeneity inevitably develops. It is impossible to estimate the time for this step (it could be hundreds of years) but, because there is no mating of BSF trypanosomes, once a minicircle class is lost it cannot be reintroduced from another trypanosome.

Step 3. A spontaneous mutation in the γ-subunit of ATP synthase eliminates the need for all kDNA-encoded products and enables the complete elimination of maxicircles. The development of minicircle homogeneity continues, and now there is no selection for retaining any of them. For example, the sequences of homogeneous minicircles of the six unique *T. equiperdum* and the four unique *T. evansi* strains that are available in GenBank (http://www.ncbi.nlm.nih.gov/GenBank/) were examined (http://rna.bmb.uga.edu/kiss/). Only one strain, a *T. equiperdum* (Accession No. V01395), encoded an A6 gRNA. The other *T. equiperdum* strains and all *T. evansi* strains encoded gRNAs for various genes (NADH dehydrogenase 3, NADH dehydrogenase 7, NADH dehydrogenase 8, NADH dehydrogenase 9, ribosomal protein S12, C-rich region 4 and cytochrome oxidase 3). These findings could indicate that the γ-subunit mutation usually occurs early in the progression to minicircle homogeneity, thus terminating the need for A6 editing. Only in *T. equiperdum* (V01395) could the γ-subunit mutation have occurred late, and A6 editing was needed until that time.

Step 4. kDNA can disappear completely, possibly because of aberrant network segregation.

establishes $\Delta\Psi$ (Figure 1c). Some yeasts, such as *Saccharomyces cerevisiae,* readily lose their mitochondrial DNA because their F_1 has sufficient ATPase activity to generate $\Delta\Psi$; other 'petite-negative' yeasts, such as *Kluyveromyces lactis,* need compensatory mutations in their α– or γ-subunits to enhance ATPase activity and, thus, enable mitochondrial-DNA-independent growth [22]. Studies on kDNA-deficient *T. brucei* and several strains of *T. equiperdum* and *T. evansi* indicate that trypanosomes resemble petite-negative yeasts and need a mutation in the γ-subunit of the ATP synthase [9,14]. The requirement for this mutation explains why the efficient drug-induced production of Ak trypanosomes rarely leads to viable cells [11]. One acriflavine-induced Ak strain required 14 passages through mice, over 102 days, to achieve stable and complete kDNA loss [23]. Box 1 shows a scenario for the step-wise loss of trypanosome kDNA.

Although it is clear that BSFs can survive without their kDNA, the reason for the dependency of PCFs on their mitochondrial genome is more puzzling. Because PCFs contain a complete repertoire of the tricarboxylic acid (TCA) cycle, electron transport chain and ATP synthase components, it was thought that both the $\Delta\Psi$ and the bulk of ATP production depended upon maxicircle-encoded proteins. However, recent experiments showed that ATP-synthase inhibition in PCFs does not substantially alter cellular ATP levels [24], which indicates that PCFs, like BSFs, use substrate-level phosphorylation to generate much of their ATP. Regardless, the electron-transport-chain-mediated generation of $\Delta\Psi$ seems to be the major reason that PCFs need kDNA. As discussed previously, if a BSF has a mutation in the γ-subunit of the ATP synthase, it can bypass the need for its kDNA and establish $\Delta\Psi$ using the F_1 portion of the ATP synthase and ADP–ATP exchange. Why can't PCFs use the same trick to eliminate their need for kDNA? Although further studies are needed to resolve this paradox, the answer is likely to lie among the myriad of metabolic differences between PCFs and BSFs. For example, dependence upon the electron transport chain for redox balance (e.g. oxidation of NADH) could contribute to an increased requirement for kDNA during trypanosome growth in the insect.

Concluding remarks

Recent discoveries concerning the structure, function and replication mechanism of kDNA have greatly clarified the biology of African trypanosomes and have been helpful in understanding the origin of Dk and Ak trypanosomes. These studies indicate that Dk and Ak trypanosomes, such as *T. evansi* and *T. equiperdum*, mirror yeast petite mutants [9,11,20]. Although the changes in kDNA have locked *T. evansi* and *T. equiperdum* in the mammalian bloodstream, they have enabled the spread of trypanosomes throughout the world, which has highlighted the incredible plasticity of this amazing parasite.

Acknowledgements

The authors thank their laboratory colleagues, Fred Opperdoes and Max Murray, for helpful discussion. National Institutes of Health grants support the Jensen laboratory (GM054021), the Simpson laboratory (AI 09102) and the Englund laboratory (AI058613).

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1471-4922/$ – see front matter
doi:10.1016/j.pt.2008.06.007 Available online 18 August 2008

Research Focus

TRAP-like protein of *Plasmodium* sporozoites: linking gliding motility to host-cell traversal

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To reach its final destination in the liver, the sporozoite (the stage of the malaria parasite that is transmitted by the mosquito vector) needs to glide through tissues and traverse host cells. Although the molecular bases of these behaviors are typically considered separately, two recent reports suggest the first molecular link between the two via a novel protein called 'TRAP-like protein'.

Gliding motility and cell traversal

The *Plasmodium* sporozoite relies on its gliding motility and host-cell traversal capacities for making its long and perilous journey from the site of injection in the skin to its final niche, the parasitophorous vacuole inside a hepatocyte. Gliding motility, a substrate-dependent type of motility, is a hallmark of the invasive stages of the Apicomplexa phylum of protozoa. By contrast, the capacity to traverse host cells (i.e. to breach their plasma membrane and glide through them) is an attribute of only a few apicomplexan invasive stages, including the *Plasmodium* sporozoite. Although the sporozoite clearly requires motility to be able to traverse host cells, the molecular machineries that drive the two processes are currently viewed as being independent. To date, the parasite molecules known to be involved in gliding motility and cell traversal are typically transmembrane proteins that bind to the motor or secreted products with membrane-lytic activity, respectively. Recent work [1,2], however, challenges this classical dichotomy with the characterization of a protein called 'TRAP-like protein' (TLP), a sporozoite motility protein that also seems to be involved specifically in cell traversal.

Sporozoites are elongated cells that glide over or across solid substrates by means of a motor running longitudinally underneath the plasma membrane. This motor, like that of other invasive apicomplexan zoites, integrates actin and a type-XIV myosin, and is sandwiched between the plasma membrane and the double inner-membrane complex, which is itself apposed on the microtubule skeleton of the zoite. The first protein that was identified as a potential link between the motor and the ligands on the substrate was TRAP (thrombospondin-related anonymous protein) of *Plasmodium* sporozoites, a surface protein that is essential for sporozoite motility [3]. TRAP has homologs in other Apicomplexa, including MIC2 (micronemal protein 2) in the *Toxoplasma* tachyzoite. The cytoplasmic tails of this family of proteins are acidic and functionally intercheangeable, and incorporate a C-terminal tryptophan residue that is crucial for zoite motility [4]. A key finding was the tryptophan-dependent interaction between the TRAP or MIC2 tails and the glycolytic enzyme aldolase [5], which itself binds to F-actin. Recently, the structure of the *P. falciparum* aldolase in complex with the C-terminal hexapeptide EDNDWN of TRAP showed that the crucial

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