J Bioenerg Biomembr (2008) 40:425–433
DOI 10.1007/s10863-008-9176-4

Mitochondria in malaria and related parasites: ancient, diverse and streamlined

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Received: 12 August 2008 / Accepted: 23 August 2008 / Published online: 24 September 2008

Abstract Parasitic organisms have emerged from nearly every corner of the eukaryotic kingdom and hence display tremendous diversity of form and function. This diversity extends to their mitochondria and mitochondrion-derived organelles. While the principles of the chemiosmotic theory apply to all these pathogens, the differences from their hosts provide opportunities for therapeutic development. In this review we discuss examples of mitochondrial systems from a deep-branching phylum, Apicomplexa. Many important human pathogens, such as malaria parasites, belong to this phylum. Unique features of their mitochondria are validated targets for drugs that are selectively toxic to the parasites.

Keywords Mitochondrial evolution · Mitochondrion-derived organelle · Human parasite · *Apicomplexa* · *Plasmodium* · *Toxoplasma* · *Cryptosporidium* · Mitochondrially-targeted drug

Introduction

With extensive investigations of mammalian and yeast mitochondrial systems, we now recognize their key role in the overall development, physiology and morphology of the cell. However, animals and fungi represent but a small fraction of the diversity of eukaryotic organisms; protists in fact comprise the largest fraction of the evolutionary range of Eucarya (Gray et al. 2004). These organisms encompass a wide variety of forms and physiologies, with a diverse set of mitochondria and mitochondrion-derived organelles. It is remarkable that no eukaryotic organism examined has yet been found to completely lack a mitochondrion or a derived organelle (Boxma et al. 2005; Dolezal et al. 2005; Dyall and Johnson 2000; Leon-Avila and Tovar 2004; Lloyd et al. 2002; Stechmann et al. 2008). Phylogenetic studies indicate that these mitochondrial forms are derivatives of a single initial endosymbiotic event (Richards and van der Giezen 2006). Also, it now appears that the key cellular function retained by nearly all mitochondrion-related organelles is not energy production nor programmed death, but biogenesis of iron–sulfur clusters (Goldberg et al. 2008; Lill and Muhlenhoff 2005; Tachezy et al. 2001; Tovar et al. 2003; Wiedemann et al. 2006).

Many significant human pathogens belong to the diverse group of eukaryotic parasites. These include the single-celled protists, such as members of *Apicomplexa*, including *Plasmodium*, the causative agent of malaria, by some measures the worst scourge on the planet (Breman et al. 2001), the opportunistic intracellular pathogen *Toxoplasma gondii* and the intestinal parasite *Cryptosporidium*; and kinetoplastids, including *Leishmania* spp. and *Trypanosoma brucei*, the agent of sleeping sickness. The metazoan group also contains many organisms that have adopted a parasitic life style, including a number of platyhelminth "flatworms" (*Schistosoma*, *Fasciola*, *Taenia*, *Hymenolepis*, etc.) and nematode "round worms" (*Ascaris*, *Brugia*, *Wuchereria*, *Necator*, *Trichuris*, etc.). Schistosomiasis is the second most prevalent tropical disease in the world, after malaria, with perhaps a quarter billion people infected (Gryseels et al. 2006). For a few recent representative

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reviews on metazoan parasites see (Bethony et al. 2006; Brooker et al. 2004; Cox 2002; Enk 2006; Gryseels et al. 2006; Hoberg 2002; Melrose 2002).

While phylogenetically, functionally and structurally diverse, all parasites seek to exploit the relative abundance of nutrients in various niches of the hosts, such as the blood with its regulated supply of glucose and other nutrients, or the intestine with frequent resupply of sugars, amino acids, and other substances courtesy of the digestive process. In adapting to maximize this advantage while minimizing metabolic costs, parasites often evolve a streamlined metabolism, even dispensing with whole pathways important in free-living organisms (Ginger 2006).

In this review we will focus on the mitochondrion or related organelle in three human parasites in the phylum *Apicomplexa*, which diverged early during eukaryotic evolution relative to metazoa and fungi. The process of metabolic streamlining in these organisms appears to include pressure to minimize the mitochondrial contribution. Yet even in the most extreme case, a reduced mitochondrion-derived organelle persists and still contains functions that could be targeted for drug development.

Morphological features of apicomplexan mitochondria

Apicomplexan parasites usually contain a single mitochondrion per cell, whose division is in synchrony with that of the nucleus. Apicomplexan mitochondria are bounded by an outer and inner membrane, but their internal structure differs considerably from that of the more familiar mammalian mitochondria. Moreover, the structure of the parasite mitochondria can vary from one stage of the life cycle to another. In addition to the mitochondrion, most apicomplexans also host a second organelle of endosymbiotic origin, a relict plastid, termed the apicoplast (McFadden et al. 1996; Wilson et al. 1996b).

Figures 1 and 2 are electron micrographs of asexual intraerythrocytic *P. falciparum* malaria parasites containing mitochondrial sections (Das et al. 1997). The internal structure of the mitochondrion is notable for the absence of cristae (Fig. 1). Other investigators reported the occasional appearance of tubular cristae (inner membrane invaginations) or membrane whorls (Fry and Beesley 1991; Learngaramkul et al. 1999; Slomianny and Prensier 1986). As the parasite matures, the mitochondrion elongates, then as the daughter nuclei begin to segregate, the mitochondrion branches, with a branch following each lobe of the dividing nucleus (Slomianny and Prensier 1986; van Dooren et al. 2005).

The single mitochondrion of *Toxoplasma gondii* tachyzoites is an elongated tubular structure extending virtually all the way around the cell periphery (Nishi et al. 2008). In contrast to *P. falciparum* asexual stages, this mitochondrion contains significant internal structure with numerous vesicular or tubular cristae (Figs. 3 and 4; Kohler 2006; Melo et al. 2000). The structure is not uniform throughout, with occasional regions largely lacking cristae. During the interphase, a domain of the mitochondrion largely devoid of the cristae is associated with the apicoplast (Kohler 2005; Nishi et al. 2008). An additional structure composed of acristate evaginations of the mitochondrion enclosing a portion of the cytoplasm has been observed in late stage tachyzoites preceding host cell lysis (Kohler 2006).

Cryptosporidium parvum sporozoites (the extracellular, infective stage of the parasite) contain an ovoid-shaped, double membrane-enclosed organelle closely apposed to the cell nucleus (Fig. 5; Keithly et al. 2005; Putignani et al. 2004); several lines of evidence indicate that this is a mitochondrion-derived organelle (see below). The organelle is enveloped by sections of rough ER extending from the outer nuclear envelope. Transmission electron microscopic and electron tomographic reconstructions demonstrated that the organelle lacks typical cristae, but contains internal membrane-delimited subcompartments (Keithly et al. 2005).

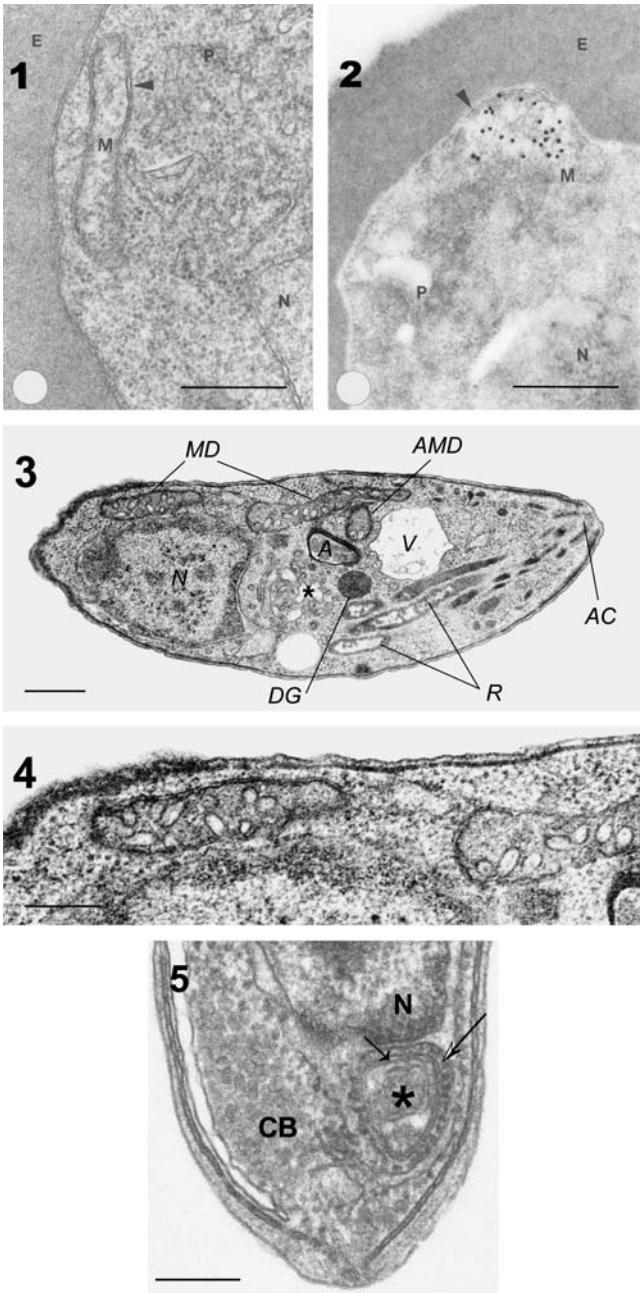
Fig. 1 Electron micrograph of Epon-embedded section of a *P. falciparum* trophozoite showing a largely acristate mitochondrial section. The arrowhead indicates a region of clearly visible outer and inner membranes. *P*, parasite; *E*, erythrocyte; *M*, mitochondrion; *N*, nucleus. *Bar*=0.5 μm. Micrograph courtesy of Nirbhay Kumar, Johns Hopkins School of Public Health, and used with permission of Elsevier Science B.V. (see Das et al. 1997)

Fig. 2 Section of a *P. falciparum* trophozoite embedded in LR white resin for immunoelectron microscopy. Anti-Hsp60 labeled gold particles are associated with a double membrane-bounded (*arrow head*) organelle. *P*, parasite; *E*, erythrocyte; *M*, mitochondrion; *N*, nucleus. *Bar*=0.5 μm. Micrograph courtesy of Nirbhay Kumar, Johns Hopkins School of Public Health, and used with permission of Elsevier Science B.V. (see Das et al. 1997)

Fig. 3 Electron micrograph of a section of an extracellular *T. gondii* tachyzoite embedded in Epon, showing sections of the mitochondrion with vesicular cristae (*MD*) and a relatively acristate mitochondrial section (*AMD*) associated with the apicoplast (*A*). Additional cellular structures visible include the nucleus (*N*), vacuole (*V*), dense granule (*DG*), rhoptries (*R*), apical complex (*AC*), and Golgi apparatus (*). Bar=0.5 μm. Micrograph courtesy of Sabine Köhler, Heinrich Heine Universität Düsseldorf

Fig. 4 Enlargement of upper left portion of Fig. 3, providing clearer view of the internal structure of the mitochondrial sections. *Bar*= 0.25 μm. Micrograph courtesy of Sabine Köhler, Heinrich Heine Universität Düsseldorf

Fig. 5 Transmission electron micrograph of the posterior end of a *C. parvum* sporozoite, displaying a mitochondrion-derived organelle (*). The small arrow indicates the delimiting double membrane, and the black/white arrow rough endoplasmic reticulum that envelops the organelle. The nucleus (*N*) and crystalloid body (*CB*) are closely adjacent. *Bar*=0.2 μm. Micrograph courtesy of Janet Keithly, Wadsworth Center, New York State Department of Health, and used with permission of Wiley-Blackwell Publishing (see Keithly et al. 2005)



Mitochondrial genome

Most apicomplexan species that infect vertebrates have a mitochondrial genome of ~6 kb, the smallest mtDNA known (Kairo et al. 1994; Vaidya and Arasu 1987; Wilson and Williamson 1997). This small genome encodes only three polytopic membrane subunits of the ETC complexes, cytochorme *b* and subunits I and III of cytochrome *c* oxidase, and ribosomal RNA genes (Feagin et al. 1992, 1997; Kairo et al. 1994; Vaidya et al. 1989). The mtDNA in *Plasmodium* is arrayed in head to tail tandem repeats (Vaidya and Arasu 1987), but in *Theileria* species it is maintained as a linear unit with terminal direct repeats (Kairo et al. 1994). Ribosomal RNA genes are present as multiple fragments arranged in a scrambled manner and present on both strands of the mtDNA. Interestingly, while the overall gene content of the mtDNA from various apicomplexan parasites is conserved, synteny is not, as the arrangement of these genes relative to each other is different. This suggests that significant mtDNA rearrangements have occurred over the period of divergence between different apicomplexan species. *Toxoplasma* represents an unusual case in which a massive number of mtDNA copies have invaded the nucleus (Ossorio et al. 1991); consequently, the nature of the authentic mtDNA is unclear. In *Cryptosporidium* species the minimalization has proceeded a step further, with the complete loss of the mtDNA (Abrahamsen et al. 2004; Putignani et al. 2004; Xu et al. 2004).

Plasmodium mitochondrial physiology and drug targets

Detailed biochemical and bioenergetic characterization of the *Plasmodium* mitochondrion has lagged, largely due to difficulties in obtaining sufficient starting material and in purifying mitochondria (Vaidya 2005). The parasites have proven to be physically tough, requiring prolonged homogenization (Fry and Beesley 1991) or high pressures (Takashima et al. 2001) to release their contents, resulting in preparations that do not consistently exhibit chemiosmotic coupling. While the mitochondrial electron transport chain (ETC) plays a central role in the oxidative energy metabolism of many types of eukaryotic cells, *Plasmodium* blood stages have a primarily glycolytic energy metabolism. Yet, they have retained ETC complexes II through IV. In place of complex I, they possess a single-subunit, non-energy-conserving NADH dehydrogenase (NDH), similar to those found in yeast and plants (see Fig. 6). Tabulation of the apparent subunit composition of the ETC complexes (Mather et al. 2007) from the genome data (Gardner et al. 2002) suggests that ETC complexes of the malaria parasite have a simpler subunit composition than the mammalian counterparts. A novel feature of apicomplexan complex IV is its split subunit II; the genes for the two parts are not found in the mitochondrial DNA, but have migrated to two different chromosomes. Measurements of oxygen consumption by infected erythrocytes, isolated parasites and mitochondrial preparations demonstrated a much lower rate of respiration in the parasites than in host mitochondria (Fry and Beesley 1991; Mather and Vaidya, unpublished). Similarly, measurement of the enzymatic activities of ETC complexes in mitochondrial preparations or with partially purified complexes (Fry and Beesley 1991; Krungkrai et al. 1997; Painter et al. 2007) found low specific activities, which perhaps correlates with the largely glycolytic carbon and energy metabolism of the blood stage parasites (see below).

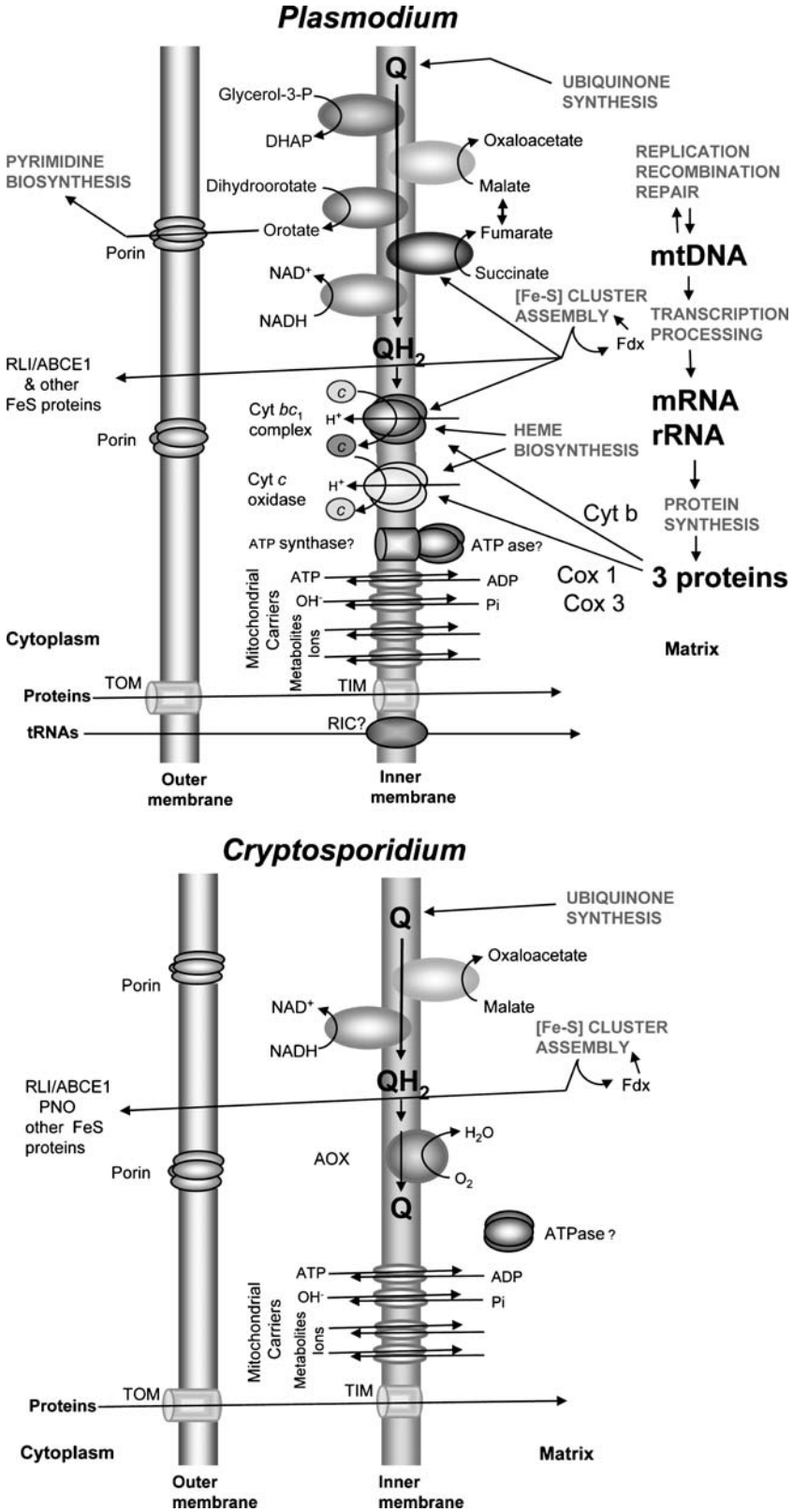


Fig. 6 Schematic illustration of mitochondrial processes in *Plasmodium* and *Cryptosporidium* parasites. An outer membrane with a putative porin allows diffusion of small molecules into and out of the intermembrane space. Transporter complexes in the inner and outer membrane assist mitochondrial import of proteins and, in the case of *Plasmodium*, tRNAs. Carriers in the inner membrane allow entry and exit of ions and metabolites. Major metabolic processes discussed in the text are indicated as upper case gray text, and the flow of substrates and products is indicated by arrows. The orientation of the dehydrogenases in the inner membrane has mostly not been demonstrated; we have shown the orientation allowing each enzyme to participate most directly in its postulated metabolic pathway

Despite the relatively low activity of the ETC in malaria parasites, it is still the primary source of the mitochondrial proton electrochemical gradient (Srivastava et al. 1997). Furthermore, the sensitivity of the parasites to inhibitors of the ETC, indicates that it is indispensible to the parasites (see following section). Hydroxynaphthoquinone inhibitors of complex III were found to be lethal to malaria parasites, leading to the development of the antimalarial drug

atovaquone, which targets the ubiquinol oxidation site (Qo) of cytochrome *b* with high selectivity (Fry and Pudney 1992; Mather et al. 2005). However, high-level resistance to atovaquone occurred at an unacceptable frequency and correlated with mutations in cytochrome *b*, specifically at tyrosine 268 in the Qo site. To improve its efficiency and lower the incidence of mutation, atovaquone is used pharmaceutically in combination with a synergistic partner drug, proguanil. We investigated the mode of action of atovaquone and proguanil using the rodent malaria *P. yoelii* and additionally using a bacterial cytochrome *b* in which a key portion of the amino acid sequence matched that of the parasite cytochrome *b* (Mather et al. 2005; Srivastava et al. 1997, 1999; Srivastava and Vaidya 1999). The results indicate that atovaquone binds in the Qo site of cytochrome *b* close to the site of interaction with the iron-sulfur subunit of the bc_1 complex, displacing ubiquinol and substantially blocking the conformational change of the iron–sulfur protein that is required for electron transfer to cytochrome c_1. This inhibition of electron transfer by submicromolar atovaquone blocks respiration and produces a concomitant reduction in mitochondrial transmembrane potential ($\Delta\psi_m$). Proguanil by itself has no effect on the ETC or $\Delta\psi_m$, but when added in combination with atovaquone, it specifically lowers the concentration of atovaquone required to dissipate $\Delta\psi_m$.

Due to the prevalence of malaria in developing areas of the world and the ability of the parasites to develop drug resistance, there is an urgent and continuing need for new, effective and inexpensive drugs. Even though complex III of the ETC is essential to both the human host and the malaria parasite, it continues to be a promising target for the development of antimalarials. Glaxo-Smith-Kline has a development program for pyridone analogs, which promise to be significantly less expensive to produce than atovaquone (Xiang et al. 2006; Yeates et al. 2008). Recently, the Riscoe group (Winter et al. 2006) produced haloalkoxyacridone derivatives that appear to target the bc_1 complex and inhibit human malaria parasite growth with IC50′s down to the picomolar range.

Ubiquinone-dependent dehydrogenases, pyrimidine biosynthesis and the essential nature of the ETC in Plasmodium Upstream of the parasite mitochondrial complex III, several dehydrogenases are known that evidently donate reducing equivalents to ubiquinone, including glycerol-3-phosphate dehydrogenase, succinate dehydrogenase, NADH dehydrogenase (NDH), malate-quinone oxidoreductase, and dihydroorotate dehydrogenase (DHODH). DHODH is part of the pyrimidine biosynthesis pathway, which is known to be essential in the parasite (Gutteridge et al. 1979), and is a target for drug development (Baldwin et al. 2005; Phillips et al. 2008). Recent results from our group have reemphasized that oxidative phosphorylation may not be an essential process in the blood stages of the human malaria parasite, but the ETC remains essential precisely to dispose of reducing equivalents from this essential enzyme (Painter et al. 2007; Vaidya et al. 2008). We generated transgenic malaria parasites expressing DHODH from yeast (Painter et al. 2007), which utilizes fumarate rather than ubiquinone and is not associated with mitochondria. The transgenic parasites proved to be resistant to all complex III inhibitors tested, that is, upon acquisition of a means to synthesize pyrimidines independent of the ETC, the ETC is no longer essential for growth of intraerythrocytic *P. falciparum* parasites. This ETC independence, however, is conditional; addition of proguanil restores the effectiveness of ETC inhibitors against the transgenic parasites. Observations with cationic fluorescent dyes suggest that this effect is related to the maintenance of $\Delta\psi_m$. In the presence of atovaquone or other ETC inhibitor alone, the potential is not fully dissipated, but together with proguanil full dissipation is produced. A possible explanation for these observations is that proguanil inhibits a secondary electrogenic process capable of maintaining a $\Delta\psi_m$ after the ETC is blocked.

Tricarboxylic acid cycle absent a pyruvate dehydrogenase complex? Data mining the genome sequence of *Plasmodium falciparum* has suggested that mitochondrial enzymes are present that may constitute a TCA cycle. However, the mere presence of the enzymes does not establish metabolic flux through a cycle, as thoroughly demonstrated in procyclic *Trypanosoma brucei* (Coustou et al. 2008; van Weelden et al. 2003, 2005). Blood stages of malarial parasites obtain energy via glycolysis and convert nearly all the pyruvate generated into lactate (regenerating NAD^+). Moreover, the pyruvate dehydrogenase complex has been localized to the apicoplast, not the mitochondrion, where it apparently supplies acetyl-CoA for fatty acid biosynthesis (Foth et al. 2005). The low level of flux through the mitochondrial ETC and its conditional dispensability also do not appear consistent with a conventional TCA cycle. Consistent with this idea, biochemical measurements of enzyme activities and recent results of gene expression studies suggest a low level of expression for several “TCA cycle” enzymes (Bozdech et al. 2003; Llinas et al. 2006; Sherman 1979, 1998; Vaidya 2005). A likely possibility is that these enzymes serve other functions, including the provision of intermediates for biosynthetic pathways, such as succinyl-CoA for heme biosynthesis (Sato and Wilson 2002; Wilson et al. 1996a). The enzyme isocitrate dehydrogenase has been proposed to serve a mitochondrial redox-balancing function, since it is NADP-linked, rather than NAD-liked, and its expression was found to be upregulated by oxidative stress (Wrenger and Muller 2003).

Other mitochondrial functions Space does not permit a full exposition of all aspects of parasite mitochondrial physiology, such as transport of small molecules, proteins and tRNA; replication; transcription; translation; iron–sulfur cluster biosynthesis; heme biosynthesis; ubiquinone biosynthesis; an unusual ATP synthase complex lacking conventional stator subunits *a* and *b*, possible amino acid catabolic pathways; involvement in Ca^{2+} homeostasis; or lack of participation in a programmed cell death pathway. We briefly discuss the important iron sulfur cluster biosynthetic pathway below. tRNA import, a process not found in animal mitochondria, is discussed in the section below on *Toxoplasma*. The interested reader should consult more complete presentations (Mather et al. 2007; Vaidya 2005; van Dooren et al. 2006) for discussion of these topics.

The one metabolic function that is present in nearly all mitochondria and mitochondrion-like organelles is the synthesis of iron–sulfur clusters (ISC). This is an ancient pathway widespread in prokaryotes and apparently essential for all eukaryotes, due, at minimum, to the ISC cofactor in the essential ABC cassette RNase L inhibitor protein required for pre-rRNA processing and ribosomal incorporation (Kispal et al. 2005; Yarunin et al. 2005). In eukaryotes, the mitochondrion is the site of primary biosynthesis, with additional components present in the cytosol to provide for insertion into cytosolic and nuclear-targeted iron–sulfur proteins (Lill and Muhlenhoff 2005, 2008). The pathway involves a number of enzymes, chaperones, and transporters; we found 18 putative proteins in *P. falciparum* (Mather et al. 2007).

Toxoplasma mitochondrial physiology

Energy metabolism, ETC, TCA cycle and oxidative phosphorylation Toxoplasma, which resides in nucleated cells, appears to be somewhat more metabolically versatile than *Plasmodium*, but the energy metabolism is still thought to be largely glycolytic, utilizing lactate dehydrogenase to regenerate NAD^+ and convert pyruvate to lactate (Coombs et al. 1997; Denton et al. 1996). Unlike malaria parasites, *Toxoplasma* contains gluconeogenic pathways, which are activated during the transition to the cyst-like bradyzoite state (Dubey et al. 1998; Fleige et al. 2008). Genomic data mining suggests that the mitochondrion contains the same ETC components and single-subunit NDH as the *Plasmodium* organelle. Like malaria parasites, *Toxoplasma* is susceptible to the complex III-targeting drug atovaquone (Araujo et al. 1991). *Toxoplasma* also appears to contain all of the TCA-cycle-like enzymes found in malaria parasites and at least one additional activity. Pyruvate carboxylase, which has not been found in *Plasmodium*, is located in the mitochondrion of tachyzoites (Fleige et al. 2008; Jelenska et al. 2001). Fleige et al. also reported that malate dehydrogenase, found in the cytoplasm in other apicomplexan parasites, was localized in the mitochondrion using a construct with a C-terminal myc epitope fusion (Fleige et al. 2008). This should be verified by an alternate method, since it is not clear that they employed the authentic start codon in this construct.

Despite the *in silico* absence of two key subunits of the ATP synthase, Vercesi et al. demonstrated apparent oligomycin-sensitive coupling of ADP phosphorylation to mitochondrial respiration in digitonin permeabilized *Toxoplasma* parasites (Vercesi et al. 1998), although the incomplete stimulation of respiration by uncouplers was puzzling. Many questions remain regarding the structure and function of complex V in apicomplexans (Mather et al. 2007; Vaidya 2005; Vaidya and Mather 2005), and further investigation may prove very enlightening.

tRNA import Mammalian mitochondrial DNA contains genes for a complete set of the tRNAs required for protein synthesis within the organelle. However, many other species have an incomplete set and require import of one or more tRNAs from the cytosol (Mirande 2007; Salinas et al. 2008). Trypanosomatid and apicommplexan parasites represent the extreme, having no tRNA genes in their mitochondrial DNA. Esseiva et al. verified the presence of nuclearly encoded tRNAs in a *Toxoplasma* mitochondrial pellet (Esseiva et al. 2004). Work with *Leishmania* has identified a large complex in the mitochondrial inner membrane responsible for binding and translocating the tRNAs (Mukherjee et al. 2007). Interestingly, the subunits of this import complex that recognize the two classes of tRNA molecules are also subunits of mitochondrial complex III (6b) and complex V (F_1-α), respectively. It will be interesting to learn whether a similar complex or other mechanism of import is responsible for tRNA import in *Plasmodium, Toxoplasma* and other apicomplexan parasites.

Physiology of the *Cryptosporidium* relict mitochondrion

Cryptosporidium parasites propagate in the microaerophilic environment of the mammalian intestine and have a glycolytic metabolism even more streamlined than *Plasmodium* spp., but with a large number of transporters to facilitate uptake of nutrients (Abrahamsen et al. 2004; Xu et al. 2004). These parasites may represent the ultimate in reductionism among the *Apicomplexa* with respect to their endosymbiont-derived organelles, having eliminated the apicoplast entirely and reduced the mitochondrion to a relict completely lacking a genome (see Fig. 6).

Mitochondrial-like chaperonins hsp60 and hsp70 were identified in *C. parvum* and found to be localized within a double membrane-enclosed organelle (Riordan et al. 2003; Slapeta and Keithly 2004). Furthermore, Roberts et al. demonstrated staining of discrete structures within intracellular trophozoite and merozoite stages of *C. parvum* by MitoTracker Green, indicative of energized mitochondrion-like organelles (Roberts et al. 2004).

The N-terminal sequences of hsp60 and hsp70 have the characteristics of mitochondrial targeting peptides, and were found to target heterologous proteins to the mitochondria in yeast and/or *T. gondii* (Riordan et al. 2003; Slapeta and Keithly 2004). A number of other putative proteins predicted to be targeted to the mitochondrial relict or to be involved in mitochondrial functions have been identified in the genomic sequences (Abrahamsen et al. 2004; Mather et al. 2007; Xu et al. 2004). These include two dehydrogenases, NDH and MQO, and a ubiquinol-dependent alternative oxidase (AOX), which together may constitute a simple alternative electron transport chain. No subunits of the ETC complexes, or TCA cycle enzymes (other than MQO) have been found, which also implies the absence of oxidative phosphorylation. Nevertheless, two subunits of complex V (F_1-α and-β) are apparently encoded in the genome; what their function may be in the absence of all the other F_1, F_o, and stator subunits is an open question.

Other apicomplexan parasites require the mitochondrial ETC to support pyrimidine biosynthesis (see discussion above and Painter et al. 2007), but *Cryptosporidium* parasites have removed this requirement by acquiring the ability to salvage host pyrimidines (Striepen et al. 2004); this may have been the key evolutionary step allowing the elimination of the ETC and thus the requirement for mitochondrial DNA. The lack of both ETC and complex V begs the question of how a mitochondrial electrochemical proton gradient is maintained in this organism (Painter et al. 2007).

As noted above, iron sulfur cluster biogenesis is a universally conserved pathway; we have noted 16 proteins likely to participate in this process in the *C. parvum* genome (Mather et al. 2007), including the core proteins cysteine desulfurase (NFS), ISU scaffold protein, ferredoxin, and ferredoxin NADPH oxidoreductase. La Gier et al. previously observed the presence of the genes encoding NFS, ISU, and ferredoxin, and noted that their sequences contained apparent N-terminal mitochondrial targeting peptides (LaGier et al. 2003). They further demonstrated that NFS and ISU are expressed in *C. parvum* sporozoites, and that the targeting peptides are functional in yeast.

The presence of ubquinone-dependent dehydrogenases and AOX indicates the need for mitochondrial ubiquinone even though the classical mitochondrial ETC is not present, and this pathway does indeed appear to have been retained in *Cryptosporidium*, while heme biosynthesis genes have been eliminated.

Presently, no effective drug treatments for cryptosporidiosis are known. The absence of the mitochondrial ETC means that classic complex III-targeting drugs such as atovaquone are not effective against *Cryptosporidium*. Suzuki et al have shown that *C. parvum* AOX is inhibited by nanomolar concentrations of ascofuranone (Suzuki et al. 2004), a substance previously shown to inhibit *T. brucei* AOX and cure *T. brucei* infected mice (Yabu et al. 2003). Thus, AOX may provide an alternative target for development of anti-*Cryptosporidium* drugs.

Concluding remarks

Here we have described a few salient points of the unusual features of mitochondrial physiology in parasitic unicellular organisms of significance to human health. These organelles provide important insights into evolutionary history of mitochondrial systems, but more importantly offer opportunities for developing therapeutics with selective toxicity against these pathogens.

Acknowledgment Writing of this review was supported by grants from NIH to ABV (AI028398 and AI053148). We are very grateful for photomicrographic materials provided by Janet Keithly, Sabine Köhler and Nirbhay Kumar.

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