CTMI (2005) 295:233–250

A Post-genomic View of the Mitochondrion in Malaria Parasites

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Abstract Mitochondria in *Plasmodium* parasites have many characteristics that distinguish them from mammalian mitochondria. Selective targeting of malaria parasite mitochondrial physiology has been exploited in successful antimalarial chemotherapy. At present, our understanding of the functions served by the parasite mitochondrion is somewhat limited, but the availability of the genomic sequences makes it possible to develop a framework of possible mitochondrial functions by providing information on genes encoding mitochondrially targeted proteins. This review aims to provide an overview of mitochondrial physiology in this post-genomic era. Although in many cases direct experimental proof for their mitochondrial functions may not be available at present, descriptions of these potential mitochondrial proteins can provide a basis for experimental approaches.

Abbreviations

GFP	Green fluorescent protein
ORF	Open reading frame

1 Introduction

The mitochondrion in *Plasmodium* parasites was deemed unusual on the basis of its acristate and 'empty' appearance in electron micrographs long before any significant biochemical studies were carried out (Aikawa 1971). The discovery of the *Plasmodium* mitochondrial DNA as the smallest such genome known with a bizarre ribosomal RNA gene arrangement further bolstered this view (Feagin et al. 1992; Vaidya et al. 1989, 1993a). This discovery also gave impetus to reassess the nature of a 35-kb circular DNA molecule present in malaria parasites that was believed to be the mitochondrial DNA. This reassessment has led to an important insight that malaria parasites (as well as most apicomplexan protozoa) possess both a mitochondrial as well as a plastid genome residing in separate organelles (Wilson et al. 1996). Both the mitochondrion and the plastid in malaria parasites are unconventional but essential for parasite physiology, and are targets for antimalarial drugs. Completion of the genomic sequence for *Plasmodium falciparum* has now opened up opportunities to explore proteins encoded in the nucleus and targeted to these organelles with a hope to gain better insight into their contribution to the parasite physiology (Gardner et al. 2002). In this review we aim to provide a view of the malaria parasite mitochondrion that is informed by bioinformatic examination of the parasite genome that permits an initial framework of the organellar physiology. While this framework, as schematically shown in Fig. 1, may not be yet fully supported by experimental proofs, it does aim to formulate testable hypotheses.

2 The Mitochondrial Genome of Malaria Parasites

At 6 kb in length, the *Plasmodium* mtDNA is the smallest such genome known among eukaryotes, encoding only three proteins (cytochrome *b*, and subunits I and III of cytochrome *c* oxidase) and ribosomal RNA encoded by fragmented genes distributed on both strands in scrambled arrangements (Feagin et al. 1992; Vaidya et al. 1989, 1993a). In light of the recently completed *Cryptosporidium* genome sequence revealing an absence of mtDNA (Abrahamsen et al. 2004; Xu et al. 2004), we could argue that the Phylum Apicomplexa is in a flux with reference to its mitochondrial genome: the minimalist mtDNA present in most members of the phylum is disposable in at least one genus. Interestingly, *Cryptosporidium* appears to maintain a vestigial organelle resembling a mitochondrion, presumably for some essential metabolic processes such as assembly of iron–sulfur clusters required for many redox proteins (LaGier et al. 2003; Riordan et al. 2003; Roberts et al. 2004).

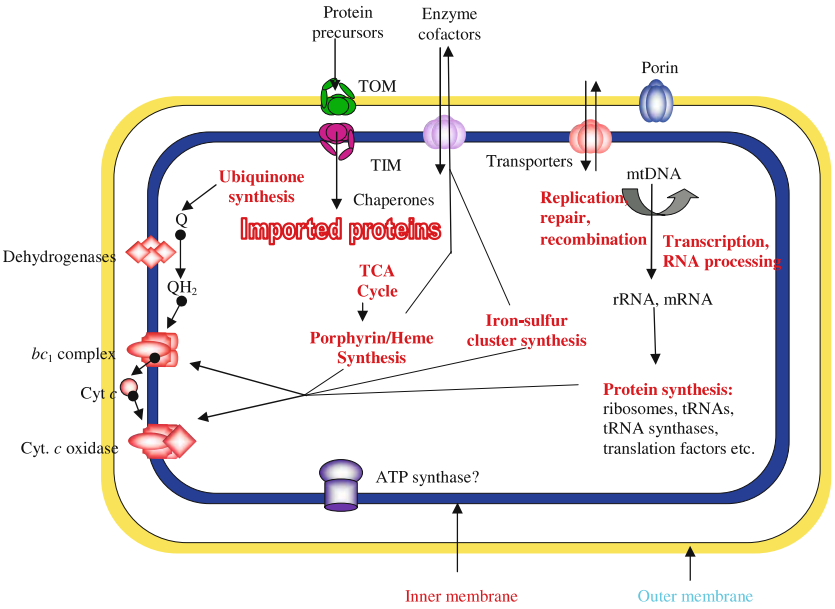

Fig. 1 A framework of mitochondrial physiology in malaria parasites. Components of the electron transport chain (described in Table 2) responsible for the generation of a proton electrochemical gradient are shown on the *left* of the figure. The mtDNA encodes only three proteins of the electron transport chain; the rest of the proteins need to be imported. Other processes relegated to the mitochondrion are shown in *red*

An unusual aspect of the malaria parasite mtDNA is its presence as multiple copies arranged in head-to-tail tandem arrays (Vaidya and Arasu 1987). This was the first example of such an arrangement for mtDNA. The number of copies appears to vary in different *Plasmodium* species, from about 30 in *P. falciparum* to about 100 in *P. yoelii*. The mtDNA does not encode any tRNA, requiring importation of the entire set of tRNA from the cytoplasm. Overall, the mtDNA is highly conserved with single nucleotide polymorphisms that can be used for establishing geographical relationships among *Plasmodium* species (Joy et al. 2003; McIntosh et al. 1998). Indeed, complete sequencing of the mtDNA from 100 *P. falciparum* isolates has provided intriguing data as to the evolutionary age of this parasite (Joy et al. 2003). The mtDNA is inherited through the female gamete during mating in the mosquito, which provides excellent markers for determining maternal lineage of progeny arising from genetic crosses between malaria parasite clones (Vaidya et al. 1993b).

3
The Challenge of Identifying Nuclearly Encoded Mitochondrial Proteins

Since the mtDNA encodes only three proteins of the mitochondrial electron transport chain, a large number of proteins encoded in the nuclear chromosomes will clearly need to be imported into parasite mitochondrion. We have made a preliminary examination of the *P. falciparum* genomic data to generate initial sets of likely and potential mitochondrial proteins (Gardner et al. 2002). Initially, we populated the list of potential mitochondrial proteins using the MitoProt II algorithm (Claros and Vincens 1996), which gives reasonably good predictions in other eukaryotes with a small number of false positives and negatives. This initial list gratifyingly included known mitochondrial proteins, such as heat shock protein 60, and many proteins that are normally mitochondrial in other organisms, such as subunits of electron transfer complexes and of the putative F-type ATP synthase. As with the *P. falciparum* genome as a whole, the number of hypothetical gene products (319) in our list outnumbered those with known or putative assignments (215). We placed the hypothetical proteins into a separate list, and culled the remainder to remove those proteins that are known or predicted to belong in other compartments. This eliminated about 48% of the remaining candidates; many of these apparent false positives were putative plastid proteins or likely cytoplasmic ribosomal proteins. To complete our working list of mitochondrial proteins, we included additional putative mitochondrial protein homologs that were in the database annotations or were reported in the literature, giving a total of 178 likely mitochondrial proteins. This list should be considered a conservative minimal catalogue of mitochondrial proteins. The list is likely to expand as proteomic data on mitochondria from other eukaryotes are used as a reference to conduct comparative genomic analysis.

Bender et al. recently developed a neural-network based program, PlasMit, specifically for predicting the mitochondrially targeted proteins of *P. falciparum* (Bender et al. 2003). They trained the neural nets using two sets of proteins that they regarded as highly likely to be targeted either to the mitochondrion or to another cellular compartment (unfortunately, there is no set of experimentally verified mitochondrial proteins in *P. falciparum* large enough to serve this purpose). Using its stringent criteria, PlasMit predicts 285 mitochondrial proteins, including hypothetical gene products. However, examination of the annotated members of this set indicates that at least 48% are probably false positives, a result similar to that given by the traditional MitoProt algorithm. From our working list of putative mitochondrial proteins generated by manual inspections informed by biochemical understanding of mitochondrial physiology in other organisms, 62% are predicted by MitoProt whereas 17% are predicted by PlasMit under stringent criteria and 65%

under nonstringent criteria. Thus, automatic predictions appear to generate unacceptable levels of false positives as well as negatives.

An experimental approach to determine mitochondrial targeting of proteins is to tag them with green fluorescent protein (GFP, or its other spectral variants) in transfection studies. The tagging is accomplished by fusing the GFP gene to the entire coding region of the protein of interest or to the putative targeting signal of the protein. Targeting of GFP fused to some of the predicted signal sequences to the malaria mitochondrion has been observed (Sato et al. 2003, 2004; Tonkin et al. 2004). The use of a strong promoter in some of these experiments, however, may compromise correct targeting as sometimes seen in other systems. Indeed, the simple act of tagging with even a small epitope has been reported to mistarget authentic mitochondrial proteins in *Saccharomyces cerevisiae* (Sickmann et al. 2003). Clearly, proteomic analysis of isolated *Plasmodium* mitochondria will be necessary to fully resolve these issues. The challenge of preparing mitochondria in acceptable purity from malaria parasites will need to be solved for this purpose.

The broad distribution of our working list of predicted mitochondrial proteins among cellular processes is shown in Fig. 2. Metabolism and translation appear to make up the bulk of the mitochondrial processes. Given the apparent lack of carbon flux through the putative tricarboxylic acid (TCA) cycle, the TCA cycle-like enzymes were assigned to anabolic, rather than catabolic, metabolism (see below). The subunits of the apparent F_1F_o ATP synthase were placed in the ambiguous group, as the function of the ATP synthase is presently uncertain (see below). In the following sections, we will discuss several of the mitochondrial processes and pathways of interest.

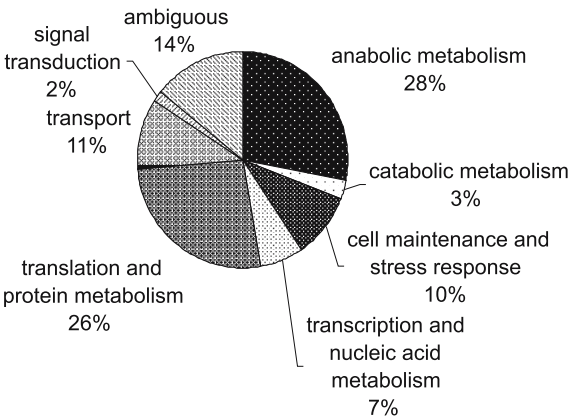

Fig. 2 Distribution among various cellular processes of 178 putative mitochondrial proteins

4
TCA Cycle Enzymes and Their Potential Roles

The TCA cycle in conjunction with oxidative phosphorylation is the central energy-yielding metabolic pathway in most aerobic organisms, and is usually localized to the mitochondrion in eukaryotes. The pathway also serves to provide intermediates to anabolic and anaplerotic pathways. Nevertheless, a number of prokaryotes and simple eukaryotes do not contain a complete TCA cycle. The existence of a conventional TCA cycle in malaria parasites has periodically been in dispute for a variety of reasons: there have been conflicting reports of the presence or absence of the activities of the various enzymes of the cycle in the blood stage forms of the parasites; ATP is derived virtually completely via glycolysis in erythrocytic stages; and the malaria genome project has revealed potential gene products corresponding to all of the enzymes required to constitute the TCA cycle (see Table 1), if expressed in the same compartment at the same time. There are also reports that some potential TCA cycle enzymes are localized in the cytoplasm rather than the mitochondrion (aconitase, Loyevsky et al. 2001; malate dehydrogenase, Lang-Unnasch 1995). The key point arguing against the presence of a canonical TCA cycle in erythrocytic stages of *P. falciparum* is the fact that essentially all of glucose consumed by the parasite is converted to lactate, depriving the TCA cycle of its key substrate, pyruvate. Furthermore, there is good evidence that the pyruvate dehydrogenase complex, which is the normal source of the input metabolite acetyl-coenzyme A for the TCA cycle, is localized to the apicoplast rather than the mitochondrion (Ralph et al. 2004). It should be remembered, however, that the TCA cycle may assume a more conventional role in other stages of the parasite life cycle, biochemical data for which are absent at this point.

Comparative and phylogenetic analyses indicate that some of the malarial enzymes are not closely related to the corresponding mitochondrial enzyme isoforms found in other eukaryotes. These include fumarate hydratase (class I) and malate-quinone oxidoreductase, which are otherwise known only in prokaryotes. The parasite malate dehydrogenase is also an isoform found predominantly in bacteria and archaea. The isocitrate dehydrogenase appears to be the $NADP^+$-dependent isoform (Chan and Sim 2003; Wrenger and Muller 2003), which in most other eukaryotes is not the isoform that participates in the TCA cycle, even though it is often located in mitochondria, its chief function being to assist in maintaining redox balance.

One hypothesis that is consistent with the apparent lack of carbon flux through the TCA cycle in erythrocytic stages is that the cycle, or portions thereof, serves primarily to generate essential metabolic intermediates, such as succinyl-CoA for the biosynthesis of heme, as alluded to in Fig. 1.

Table 1 Potential TCA-cycle enzymes in *P. falciparum*

Enzyme	PlasmoDB ID	Length (aa)	MitoP score[a]	PlasMit prediction[b]
Citrate synthase	PF10_0218	563	0.9	+/−
Aconitase/IRP	PF13_0229	909	0.95	+
Isocitrate dehydrogenase	PF13_0242	468	0.9	+
α-Ketoglutarate dehydrogenase				
E1 subunit	PF08_0045	1038	0.95	−
E2 subunit	PF13_0121	421	0.94	+/−
Succinyl-CoA synthase				
SCSa	PF11_0097	327	0.09	+
SCSb	PF14_0295	462	0.9	+/−
Succinate dehydrogenase				
SDHa	PF10_0334	631	0.91	+
SDHb	PFL0630w	321	0.13	+
Fumarate hydratase	PFI1340w	681	0.8	−
Malate dehydrogenase[c]	PFF0895w	313	0.07	−
Malate quinone oxidoreductase	PFF0815w	521	0.3	+

[a]Predicted probability that the protein is likely targeted to the mitochondrion.
[b]+, Indicates mitochondrial localization prediction under stringent criteria; +/− indicates mitochondrial localization prediction under relaxed criteria; −, indicates mitochondrial localization not predicted.
[c]Malate dehydrogenase is localized to the cytosol.

5 The Mitochondrial Electron Transport Chain

In most eukaryotes, the electron transport chain is a key part of the cell's energy transduction machinery, transferring the reducing equivalents from catabolic metabolism to oxygen in a series of energy-conserving steps that establish a transmembrane proton gradient across the mitochondrial inner membrane. The proton gradient is used to power the production of the majority of ATP in metabolically active cells, as well as to assist mitochon-

drial transport and other processes. In metazoans, the electron transport chain is composed of four integral membrane enzyme complexes in the mitochondrial inner membrane, NADH-ubiquinone oxidoreductase (complex I), succinate-ubiquinone oxidoreductase (complex II), ubiquinol-cytochrome *c* oxidodreductase (complex III), and cytochrome *c* oxidase (complex IV), plus ubiquinone (Coenzyme Q) and cytochrome *c*, which function as electron carriers completing the electron circuit between the complexes. Complexes I, III, and IV catalyze energy-conserving steps, coupling electron transfer to transmembrane proton translocation. While the mitochondrion is not a significant source of ATP biosynthesis in *Plasmodium*, the electron transport is still essential, probably for at least two reasons: (1) to provide an electron sink for ubiquinone-dependent dehydrogenases required for cellular metabolism, such as dihydroorotate dehydrogenase (Gutteridge et al. 1979), succinate dehydrogenase, and glycerol-3-phosphate dehydrogenase and (2) to energize the transmembrane proton gradient required for the transport of metabolites and proteins across the mitochondrial membranes. In addition to the dehydrogenases mentioned above, genomic data indicate the presence of a novel malate-quinone oxidoreductase, previously unknown in eukaryotes, which may replace NAD-dependent malate dehydrogenase in the mitochondrion, although its physiological importance has yet to be investigated. A comparison of mammalian and *Plasmodium* enzymes involved in electron transport chain is given in Table 2.

The *Plasmodium* electron transport chain differs from the classical chain in lacking a complex I; however, a single subunit, nonenergy-conserving NADH dehydrogenase is present, which is homologous to peripheral membrane NADH dehydrogenases found in yeast, plants, and many bacteria, but not in animals (Luttik et al. 1998; Yagi et al. 2001). *P. falciparum* may also lack an integral membrane complex II as homologues of the membrane anchor subunits of complex II are not evident in the genome, but it does express a peripheral membrane succinate dehydrogenase (Suraveratum et al. 2000; Takeo et al. 2000).

The 'business' subunits of *Plasmodium* complex III (cytochrome *b*, cytochrome c_1, and the Rieske iron sulfur protein) are homologous to their corresponding mammalian orthologs, and the complex is inhibited by classical complex III inhibitors, such as myxothiazol and antimycin A, which are ubiquinol/ubiquinone antagonists. As we have previously noted, there are differences in the ubiquinol ('Q_o') binding region of cytochrome *b* of the *Plasmodium* complex III versus the mammalian cytochrome *b* (Vaidya et al. 1993a). These unique structural features are the basis for the selective toxicity of the potent antimalarial drug, atovaquone (Srivastava et al. 1997, 1999). The development and mode of action of atovaquone have been reviewed elsewhere (Vaidya 1998, 2004). The mammalian complex III contains

Table 2 Comparison of mitochondrial electron transport chain enzymes in mammals and *Plasmodium*

Enzyme	Mammalian	*Plasmodium*	Comments
NADH dehydrogenase	42–44 subunits	Single subunit	The single subunit enzyme of malaria parasites does not have an ortholog in mammals; many plants, fungi and bacteria have orthologs
Succinate dehydrogenase	4 subunits	2 subunits	Membrane anchoring subunits not detected in the parasite genome
Dihydroorotate dehydrogenase	Single subunit	Single subunit	Differential susceptibility of the mammalian and parasite enzymes to inhibitors (Baldwin et al. 2002), suggesting a therapeutic window
Malate dehydrogenase	Single subunit	Single subunit	Cytosolic localization in the parasite; mitochondrial isozyme likely replaced by malate quinone oxidoreductase (see below)
Glycerol 3-phosphate dehydrogenase	Single subunit	Single subunit	Ubiquinone is the electron acceptor
Malate quinone oxidoreductase	Absent	Single subunit	First eukaryote to possess this otherwise prokaryotic enzyme. Not seen in mammals
Ubiquinone cytochrome *c* oxidoreductase	11 subunits	7 subunits	Subtle structural difference of cytochrome *b* is the likely reason for this enzyme being a validated antimalarial target
Cytochrome *c* oxidase	13 subunits	5 subunits	Appears to have significantly streamlined subunit composition. Subunit II is encoded by two genes
F_1F_o ATP synthase	9 subunits	7 subunits	Subunits a and b of the F_o segment cannot be detected in the genome. Functionality is questioned

eight additional subunits in addition to the three containing the cofactors and ubiquinone/ubiquinol binding sites, whereas the yeast complex and several plant complexes have seven such subunits. Putative homologs for subunit I (MPPβ/core 1; PFI1625c), subunit II (MPPα/core 2; PFE1155c), subunit VII

(QPc; PF10_0120), and subunit VIII (hinge protein; PF14_0248) have been identified in the Pf genome database. The *Plasmodium* complex III, then, probably has a subunit composition similar to or slightly simpler than the complexes from yeast and plants, with some of the smaller subunits undetected in the genome database due to their short length and/or relatively low degree of conservation. There appears to be only one set of genes coding for putative Complex III 'core' 1 and 2 subunits and for the mitochondrial processing peptidase α and β subunits. Thus, the processing peptidase probably serves dual functions both as a processing enzyme and as the 'core' subunits of complex III, as reported for plants and some other lower eukaryotes (Brumme et al. 1998).

Complex IV, or cytochrome *c* oxidase, is the terminal enzyme complex of the mitochondrial electron transport chain, in which the electrons are finally donated to dioxygen in a strongly exergonic reaction producing water. The complex uses part of the energy of this reaction to 'pump' up to two protons per pair of electrons across the plane of the inner membrane, helping to establish the electrochemical proton gradient. The mammalian complex consists of 13 subunits, while simpler eukaryotes generally have fewer subunits (e.g., nine in yeast, six in *Dictyostelium*). Subunits I and II contain the active centers for electron transfer as well as oxygen binding and reduction. In most organisms, they are encoded by the mtDNA, along with subunit III. The remaining smaller subunits, some of which have regulatory functions, are encoded in the nucleus. In *Plasmodium*, subunits I and III are encoded by the mtDNA, but the subunit II gene has been transferred to the nucleus and split into two parts in the process (PF13_0327, PF14_0288). Until now, this was observed only in the Chlamydomonad algae family (Perez-Martinez et al. 2001), which suggests the possibility that the cytochrome *c* oxidase subunit II originated in the mitochondrion of the algal endosymbiont, rather than in the ancestral protist mitochondrion. Only two additional putative oxidase subunits were detected in the genome (PFI1365w, PFI1375w), apparent orthologs of mammalian subunits Vb and VIb. Thus, *Plasmodium* cytochrome *c* oxidase appears to have the most streamlined subunit composition among the eukaryotes.

6 The Mystery of F_1F_o-ATP Synthase

F_1F_o-ATP synthase (Complex V) completes the process of oxidative phosphorylation by using the energy stored in the transmembrane proton gradient to drive the endergonic synthesis of ATP from ADP and inorganic phosphate. The ATP synthase/ATPase functions as a reversible protonic motor (Boyer 2001); if the proton gradient has been depleted and ATP is available, it will

use energy from the hydrolysis of ATP to translocate protons from the mitochondrial matrix across the inner membrane, regenerating the gradient. An F_1F_o-ATP synthase is a component of the inner membrane of mitochondria in all cases that have been characterized, and is also found in chloroplasts and in bacteria. The F_1 sector of the complex is detachable from the membrane under relatively mild conditions and consists of α (three copies), β (three copies), γ, δ, ε, and oligomycin sensitivity conferring protein (OSCP) subunits. The F_o subcomplex is integral to the membrane, where it acts as a proton channel consisting of three subunits: a, b, and ~12 copies of c.

The *P. falciparum* genome contains putative subunits corresponding to all of the expected ATP synthase subunits, except for the F_o a and b subunits. Since a small portion of the *P. falciparum* genome was refractory to sequencing, it is possible that these subunits are encoded in the unsequenced DNA regions. This is unlikely, however, due to the similar inability to detect a or b subunit homology in the genome of any other *Plasmodium* species, including *P. yoelii*, which was sequenced at an average coverage of five times (Carlton et al. 2002); nor could they be detected in the large body of genomic/cDNA/EST data from a number of other *Plasmodium* species. Subunit a is essential for F_1F_o ATP synthase function. Protons from the matrix channel through a to the c subunits, the latter forming a ring that rotates in the membrane. This rotational movement of the c subunit ring is linked to the central stalk of the F_1 portion of the enzyme, and ultimately to the synthesis of ATP at the active site in one of the β subunits. We are not aware of any report of the confirmed absence of these subunits in any species. Thus, the apparent absence of these subunits is puzzling. One possibility is that the functions of the missing subunits may be served by recruitment of novel polypeptides. It is also possible that the ATP synthase subunits may serve unconventional functions. Seeking the solution to this mystery will be a very worthwhile endeavor.

7
Replication, Transcription, and Translation of mtDNA

Except for the fragmented rRNA molecules, all of the components necessary for replication, transcription and translation of mtDNA will need to be encoded in the nucleus for transport to the mitochondrion. Based on morphological and electrophoretic properties of mtDNA, Preiser et al. proposed a rolling circle mode of replication with extensive gene conversion and recombination for the parasite mtDNA (Preiser et al. 1996). This would require participation of a large number of proteins such as those involved in the complex process of recombination, in addition to the usual DNA replication and repair

enzymes. At present, little is known about these proteins in malaria parasites. The genome sequence failed to reveal the presence of DNA polymerase γ, the canonical mitochondrial DNA polymerase. However, there are two genes encoding proteins with homology to DNA polymerase I. PF14_0112, currently annotated as POM1, encodes a 2,016 amino acid open reading frame (ORF) with homology to DNA polymerase I at its C terminus, which is preceded by regions with homology to a $3' \rightarrow 5'$ exonuclease and a DNA helicase/primase. The predicted protein also has an apicoplast targeting signal at its N terminus, and the protein appears to be post-translationally processed (Michael Barrett et al. personal communication). PFF1225c encodes a1, 444-amino acid ORF with a DNA polymerase domain located at the C-terminal region. Further investigations on these gene products will be informative as to their functions and localization.

The mtDNA is transcribed in a complex manner, generating almost 20 discrete stable RNA molecules (Feagin et al. 1992; Ji et al. 1996; Suplick et al. 1990). Three of these are mRNAs encoding the proteins, whereas the rest are fragments of rRNA or apparent precursors from which these rRNA fragments are generated. An RNA polymerase with similarity to bacteriophage T3/T7 RNA polymerase has been identified, encoded by the PF11_0264 gene (Li et al. 2001). This protein of 1,531 amino acids is likely to be the polymerase that transcribes the parasite mtDNA. Other components of the mitochondrial transcription machinery have not yet been identified.

The mitochondrial ribosomes in malaria parasites have a highly unusual organization: multiple rRNA fragments will need to interact in trans to form the core small and large subunit rRNA structures, which will in turn interact with imported ribosomal proteins to form functional ribosomes (Feagin et al. 1992; Vaidya et al. 1993a). At present, little is known about the components and the process underlying the assembly of these bizarre ribosomes. Through a bioinformatics approach, a number of nuclearly encoded putative mitochondrial ribosomal proteins have been identified, and the N-terminal signal of one has been experimentally shown to target a fused GFP to the mitochondrion (Perrault and Vaidya, unpublished data). The parasite mtDNA does not encode any tRNAs or tRNA synthetases, all of which will need to be imported. Genomic sequences do not reveal any tRNA genes other than those for cytoplasmic protein synthesis, thus tRNAs will require dual targeting in malaria parasites. Indeed, there do not appear to be sufficient tRNA synthetase genes to independently serve the three compartments–the cytoplasm, the mitochondrion and the apicoplast–in which protein synthesis occurs in malaria parasites. Hence some of the tRNA synthetases will have dual or triple targeting. A similar situation is likely to exist for a number of different components required for protein synthesis.

8
Metabolic Functions of the Mitochondrion

Although the mitochondrion does not appear to be a source of ATP in erythrocytic stages of malaria parasites, it does serve essential functions in several metabolic processes. A major function proposed originally by Gutteridge et al. (1979) is in de novo pyrimidine biosynthesis by serving as the electron sink for dihydroorotate dehydrogenase, which is present within the mitochondrion and uses ubiquinone as the electron acceptor. Since malaria parasites are incapable of pyrimidine salvage and rely solely on de novo synthesis, interference with mitochondrial functions will have a deleterious effect by limiting the pyrimidine supply. Indeed, mitochondrial electron transport is a validated target for antimalarial drugs such as atovaquone (Fry and Pudney 1992; Srivastava et al. 1997).

Heme biosynthesis in many eukaryotes is initiated within the mitochondrion, and malaria parasites encode δ-aminolevulinate synthase, the rate limiting enzyme in heme synthesis, targeted to the mitochondrion (Sato et al. 2004). Whereas in most other organisms several of the subsequent steps in heme biosynthesis occur in the cytosol, in malaria parasites these steps appear to be relegated to the apicoplast (Dhanasekaran et al. 2004; Foth and McFadden 2003; Sato et al. 2004; Wilson 2002). Ferrochelatase and heme lyases, the last set of enzymes in hemoprotein assembly, are usually located within the mitochondrion. A recent report, however, suggests apicoplast localization of the parasite-encoded ferrochelatase (Varadharajan et al. 2004). Malaria parasites possess a-, b- and c-type cytochromes (present in the respiratory chain) and possibly also a cytochrome P450 (as inferred from the presence of a gene encoding cytochrome P450 reductase, although a conventional P450 monooxygenase cannot be detected). Thus, the mitochondrion will be critical in assembly of these proteins.

Iron-sulfur [Fe–S] clusters are components of many proteins in which they participate in a variety of different ways, such as electron transfer reactions, gene regulation and sensing of environmental signals (Beinert 2000). Malaria parasites also possess many [Fe–S] proteins. Biogenesis of [Fe–S] clusters is a complex process requiring participation of several enzymes and chaperones, often present within the mitochondria (Lill and Kispal 2000). Indeed, many amitochondriate protozoa appear to possess vestigial mitochondrion-like organelles to which components of [Fe–S] assembly are localized (Seeber 2002). In *P. falciparum,* components for [Fe–S] cluster generation have been identified to be targeted to the apicoplast (Ellis et al. 2001; Seeber 2002). However, the genomic sequence also reveals another set of genes that may encode [Fe–S] cluster generating proteins that may be targeted to the mitochondrion. For example, elemental sulfur needed for [Fe–S] clusters is generated by cys-

teine desufurase; the *P. falciparum* genome has one gene that may target the protein to the apicoplast (PF07_0068) and another that may be specific for the mitochondrion (MAL7P1.150), both present on chromosome 7.

Close collaboration between the mitochondrion, apicoplast and the cytoplasm would clearly require the activity of a number of transporters for the exchange of metabolites and precursors. Since almost all of the proteins necessary for mitochondrial functions are imported from the cytoplasm, protein transporters located within the outer and inner mitochondrial membranes (TOMs and TIMs) will clearly be required, and the genome reveals several candidate components of these complexes. In other eukaryotes, mitochondria play a significant role in calcium homeostasis. And one can envision a similar role for the organelle in malaria parasites as well. Appropriate import of other cations, such as Cu and Fe, as well as their appropriate assembly into metalloproteins, are also likely to be critical features of the mitochondrial physiology in malaria parasites. At present, the identity of proteins that carry out metal transport in mitochondria is not clear.

9 Perspectives

The mitochondrion of malaria parasites clearly has unconventional features. The genome has revealed sequences that are likely to be critical for mitochondrial contributions to the parasite physiology. That the mitochondrial physiology is a validated target for antimalarial chemotherapy has been amply proven by the action of atovaquone. The novel features of the mitochondrially targeted sequences need closer examination, which may provide leads for development of antimalarial compounds as well as a better understanding of a minimalist mitochondrion.

Acknowledgements We thank our colleagues for helpful discussions. This work was supported by a grant (AI028398) from the National Institutes of Health.

References

Abrahamsen MS, Templeton TJ, Enomoto S, Abrahante JE, Zhu G, Lancto CA, Deng M, Liu C, Widmer G, Tzipori S, Buck GA, Xu P, Bankier AT, Dear PH, Konfortov BA, Spriggs HF, Iyer L, Anantharaman V, Aravind L, Kapur V (2004) Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. Science 304**:**441–445

Aikawa M (1971) Parasitological review. *Plasmodium*: the fine structure of malarial parasites. Exp Parasitol 30**:**284–320

Baldwin J, Farajallah AM, Malmquist NA, Rathod PK, Phillips MA (2002) Malarial dihydroorotate dehydrogenase. Substrate and inhibitor specificity. J Biol Chem 277:41827–41834

Beinert H (2000) Iron-sulfur proteins: ancient structures, still full of surprises. J Biol Inorg Chem 5:2–15

Bender A, van Dooren GG, Ralph SA, McFadden GI, Schneider G (2003) Properties and prediction of mitochondrial transit peptides from *Plasmodium falciparum*. Mol Biochem Parasitol 132:59–66

Boyer PD (2001) New insights into one of nature's remarkable catalysts, the ATP synthase. Mol Cell 8:246–247

Brumme S, Kruft V, Schmitz UK, Braun HP (1998) New insights into the co-evolution of cytochrome c reductase and the mitochondrial processing peptidase. J Biol Chem 273:13143–13149

Carlton JM, Angiuoli SV, Suh BB, Kooij TW, Pertea M, Silva JC, Ermolaeva MD, Allen JE, Selengut JD, Koo HL, Peterson JD, Pop M, Kosack DS, Shumway MF, Bidwell SL, Shallom SJ, van Aken SE, Riedmuller SB, Feldblyum TV, Cho JK, Quackenbush J, Sedegah M, Shoaibi A, Cummings LM, Florens L, Yates JR, Raine JD, Sinden RE, Harris MA, Cunningham DA, Preiser PR, Bergman LW, Vaidya AB, van Lin LH, Janse CJ, Waters AP, Smith HO, White OR, Salzberg SL, Venter JC, Fraser CM, Hoffman SL, Gardner MJ, Carucci DJ (2002) Genome sequence and comparative analysis of the model rodent malaria parasite Plasmodium *yoelii yoelii*. Nature 419:512–519

Chan M, Sim TS (2003) Recombinant *Plasmodium falciparum* NADP-dependent isocitrate dehydrogenase is active and harbours a unique 26 amino acid tail. Exp Parasitol 103:120–126

Claros MG, Vincens P (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. Eur J Biochem 241:779–786

Dhanasekaran S, Chandra, NR, Chandrasekhar Sagar, BK, Rangarajan PN, Padmanaban G (2004) Delta-aminolevulinic acid dehydratase from *Plasmodium falciparum*: indigenous versus imported. J Biol Chem 279:6934–6942

Ellis KE, Clough B, Saldanha JW, Wilson RJ (2001) Nifs and Sufs in malaria. Mol Microbiol 41:973–981

Feagin JE, Werner E, Gardner MJ, Williamson DH, Wilson RJ (1992) Homologies between the contiguous and fragmented rRNAs of the two *Plasmodium falciparum* extrachromosomal DNAs are limited to core sequences. Nucleic Acids Res 20:879–887

Foth BJ, McFadden GI (2003) The apicoplast: a plastid in *Plasmodium falciparum* and other Apicomplexan parasites. Int Rev Cytol 224:57–110

Fry M, Pudney M (1992) Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(4'-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone (566C80). Biochem Pharmacol 43:1545–1553

Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan MS, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DM, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419:498–511

Gutteridge WE, Dave D, Richards WH (1979) Conversion of dihydroorotate to orotate in parasitic protozoa. Biochim Biophys Acta 582:390–401

Ji YE, Mericle BL, Rehkopf DH, Anderson JD, Feagin JE (1996) The *Plasmodium falciparum* 6 kb element is polycistronically transcribed. Mol Biochem Parasitol 81:211–223

Joy DA, Feng X, Mu J, Furuya T, Chotivanich K, Krettli AU, Ho M, Wang A, White NJ, Suh E, Beerli P, Su XZ (2003) Early origin and recent expansion of *Plasmodium falciparum*. Science 300:318–321

LaGier MJ, Tachezy J, Stejskal F, Kutisova K, Keithly JS (2003) Mitochondrial-type iron-sulfur cluster biosynthesis genes (IscS and IscU) in the apicomplexan *Cryptosporidium parvum*. Microbiology 149: 3519–3530

Lang-Unnasch N (1995) *Plasmodium falciparum*: antiserum to malate dehydrogenase. Exp Parasitol 80:357–359

Li J, Maga JA, Cermakian N, Cedergren R, Feagin JE (2001) Identification and characterization of a *Plasmodium falciparum* RNA polymerase gene with similarity to mitochondrial RNA polymerases. Mol Biochem Parasitol 113:261–269

Lill R, Kispal G (2000) Maturation of cellular Fe-S proteins: an essential function of mitochondria. Trends Biochem Sci 25:352–356

Loyevsky M, LaVaute T, Allerson CR, Stearman R, Kassim OO, Cooperman S, Gordeuk VR, Rouault TA (2001) An IRP-like protein from *Plasmodium falciparum* binds to a mammalian iron-responsive element. Blood 98:2555–2562

Luttik MA, Overkamp KM, Kotter P, de Vries S, van Dijken JP, Pronk JT (1998) The *Saccharomyces cerevisiae* NDE1 and NDE2 genes encode separate mitochondrial NADH dehydrogenases catalyzing the oxidation of cytosolic NADH. J Biol Chem 273:24529–24534

McIntosh MT, Srivastava R, Vaidya AB (1998) Divergent evolutionary constraints on mitochondrial and nuclear genomes of malaria parasites. Mol Biochem Parasitol 95:69–80

Perez-Martinez X, Antaramian A, Vazquez-Acevedo M, Funes S, Tolkunova E, d'Alayer J, Claros MG, Davidson E, King MP, Gonzalez-Halphen D (2001) Subunit II of cytochrome c oxidase in Chlamydomonad algae is a heterodimer encoded by two independent nuclear genes. J Biol Chem 276:11302–11309

Preiser PR, Wilson RJ, Moore PW, McCready S, Hajibagheri MA, Blight KJ, Strath M, Williamson DH (1996) Recombination associated with replication of malarial mitochondrial DNA. EMBO J 15: 684–693

Ralph SA, Van Dooren GG, Waller RF, Crawford MJ, Fraunholz MJ, Foth BJ, Tonkin CJ, Roos DS, McFadden GI (2004) Tropical infectious diseases: Metabolic maps and functions of the *Plasmodium falciparum* apicoplast. Nat Rev Microbiol 2:203–216

Riordan CE, Ault JG, Langreth SG, Keithly JS (2003) *Cryptosporidium parvum* Cpn60 targets a relict organelle. Curr Genet 44:138–147

Roberts CW, Roberts F, Henriquez FL, Akiyoshi D, Samuel BU, Richards TA, Milhous W, Kyle D, McIntosh L, Hill GC, Chaudhuri M, Tzipori S, McLeod R (2004) Evidence for mitochondrial-derived alternative oxidase in the apicomplexan parasite *Cryptosporidium parvum*: a potential anti-microbial agent target. Int J Parasitol 34:297–308

Sato S, Clough B, Coates L, Wilson RJ (2004) Enzymes for heme biosynthesis are found in both the mitochondrion and plastid of the malaria parasite *Plasmodium falciparum*. Protist 155:117–125

Sato S, Rangachari K, Wilson RJ (2003) Targeting GFP to the malarial mitochondrion. Mol Biochem Parasitol 130:155–158

Seeber F (2002) Biogenesis of iron-sulphur clusters in amitochondriate and apicomplexan protists. Int J Parasitol 32:1207–1217

Sickmann A, Reinders J, Wagner Y, Joppich C, Zahedi R, Meyer HE, Schonfisch B, Perschil I, Chacinska A, Guiard B, Rehling P, Pfanner N, Meisinger C (2003) The proteome of *Saccharomyces cerevisiae* mitochondria. Proc Natl Acad Sci USA 100:13207–13212

Srivastava IK, Morrisey JM, Darrouzet E, Daldal F, Vaidya AB (1999) Resistance mutations reveal the atovaquone-binding domain of cytochrome b in malaria parasites. Mol Microbiol 33:704–711

Srivastava IK, Rottenberg H, Vaidya AB (1997) Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. J Biol Chem 272:3961–3966

Suplick K, Morrisey J, Vaidya AB (1990) Complex transcription from the extrachromosomal DNA encoding mitochondrial functions of *Plasmodium yoelii*. Mol Cell Biol 10:6381–6388

Suraveratum N, Krungkrai SR, Leangaramgul P, Prapunwattana P, Krungkrai J (2000) Purification and characterization of *Plasmodium falciparum* succinate dehydrogenase. Mol Biochem Parasitol 105:215–222

Takeo S, Kokaze A, Ng CS, Mizuchi D, Watanabe JI, Tanabe K, Kojima S, Kita K (2000) Succinate dehydrogenase in *Plasmodium falciparum* mitochondria: molecular characterization of the SDHA and SDHB genes for the catalytic subunits, the flavoprotein (Fp) and iron-sulfur (Ip) subunits. Mol Biochem Parasitol 107:191–205

Tonkin CJ, van Dooren GG, Spurck TP, Struck NS, Good RT, Handman E, Cowman AF, McFadden GI (2004) Localization of organellar proteins in *Plasmodium falciparum* using a novel set of transfection vectors and a new immunofluorescence fixation method. Mol Biochem Parasitol 137:13–21

Vaidya AB (1998) Mitochondrial physiology as a target for atovaquone and other antimalarials. In: Sherman IW (ed.) Malaria: Parasite biology, pathogenesis, and protection. ASM Press, Washington DC, pp 355–368

Vaidya AB (2004) Mitochondrial and plastid functions as antimalarial drug targets. Curr Drug Targets Infect Disord 4:11–23

Vaidya AB, Akella R, Suplick K (1989) Sequences similar to genes for two mitochondrial proteins and portions of ribosomal RNA in tandemly arrayed 6-kilobase-pair DNA of a malarial parasite. Mol Biochem Parasitol 35:97–107

Vaidya AB, Arasu P (1987) Tandemly arranged gene clusters of malarial parasites that are highly conserved and transcribed. Mol Biochem Parasitol 22:249–257

Vaidya AB, Lashgari MS, Pologe LG, Morrisey J (1993a) Structural features of *Plasmodium* cytochrome b that may underlie susceptibility to 8-aminoquinolines and hydroxynaphthoquinones. Mol Biochem Parasitol 58:33–42

Vaidya AB, Morrisey J, Plowe CV, Kaslow DC, Wellems TE (1993b) Unidirectional dominance of cytoplasmic inheritance in two genetic crosses of *Plasmodium falciparum*. Mol Cell Biol 13:7349–7357

Varadharajan S, Sagar BK, Rangarajan PN, Padmanaban G (2004) Localization of ferrochelatase in *Plasmodium falciparum*. Biochem J 384:429–436

Wilson RJ (2002) Progress with parasite plastids. J Mol Biol 319:257–274

Wilson RJ, Denny PW, Preiser PR, Rangachari K, Roberts K, Roy A, Whyte A, Strath M, Moore DJ, Moore PW, Williamson DH (1996) Complete gene map of the plastid-like DNA of the malaria parasite *Plasmodium falciparum*. J Mol Biol 261**:**155–172

Wrenger C, Muller S (2003) Isocitrate dehydrogenase of *Plasmodium falciparum*. Eur J Biochem 270**:**1775–1783

Xu P, Widmer G, Wang Y, Ozaki LS, Alves JM, Serrano MG, Puiu D, Manque P, Akiyoshi D, Mackey AJ, Pearson WR, Dear PH, Bankier AT, Peterson DL, Abrahamsen MS, Kapur V, Tzipori S, Buck GA (2004) The genome of *Cryptosporidium hominis*. Nature 431**:**1107–1112

Yagi T, Seo BB, Di Bernardo S, Nakamaru-Ogiso E, Kao MC, Matsuno-Yagi A (2001) NADH dehydrogenases: from basic science to biomedicine. J Bioenerg Biomembr 33**:**233–242