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# The metabolic roles of the endosymbiotic organelles of *Toxoplasma* and *Plasmodium* spp.

Lilach Sheiner[1,2], Akhil B Vaidya[3] and Geoffrey I McFadden[4]

The apicoplast and the mitochondrion of Apicomplexa cooperate in providing essential metabolites. Their co-evolution during the ancestral acquisition of a plastid and subsequent loss of photosynthesis resulted in divergent metabolic pathways compared with mammals and plants. This is most evident in their chimerical haem synthesis pathway.*Toxoplasma* and *Plasmodium* mitochondria operate canonical tricarboxylic acid (TCA) cycles and electron transport chains, although the roles differ between *Toxoplasma* tachyzoites and *Plasmodium* erythrocytic stages. Glutamine catabolism provides TCA intermediates in both parasites.Isoprenoid precursor synthesis is the only essential role of the apicoplast in *Plasmodium* erythrocytic stages. An apicoplast-located fatty acid synthesis is dispensable in these stages, which instead predominantly salvage fatty acids, while in *Plasmodium* liver stages and in *Toxoplasma* tachyzoites fatty acid synthesis is an essential role of the plastid.

**Addresses**
[1] Center for Tropical and Emerging Global Diseases & Department of Cellular Biology, University of Georgia, 500 D.W. Brooks Drive, Athens, GA 30602, USA
[2] Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Sciences, Sir Graeme Davies Building, University of Glasgow, 120 University Place, Glasgow G12 8TA, United Kingdom
[3] Center for Molecular Parasitology and Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19129, USA
[4] School of Botany, The University of Melbourne, Parkville, Vic 3010, Australia

Corresponding author: Sheiner, Lilach (lilash@uga.edu)

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## Introduction

Apicomplexan parasites possess two organelles of endosymbiotic origin: a relict non-photosynthetic plastid (the apicoplast), and a mitochondrion (Figure 1), which together contribute substantially to the parasites' metabolic needs. The apicoplast and mitochondrion show tight physical [1,2] and functional collaboration. A chimerical haem pathway spans both organelles [3]. Apicoplast generated isopentenyl pyrophosphate (IPP) is likely used in mitochondrion co-enzyme Q synthesis, and finally the *Toxoplasma* mitochondrion and apicoplast shared a citrate shunt [4••].

In accordance with the adaptation of each parasite to its specific host niche, the repertoire of apicoplast and mitochondrion metabolic pathways has diverged between the different phylum members [5]. Here we focus on the unique features of these pathways in *Plasmodium* and *Toxoplasma* and review our current understanding of their roles in different host environments.

## The apicomplexan mitochondrion

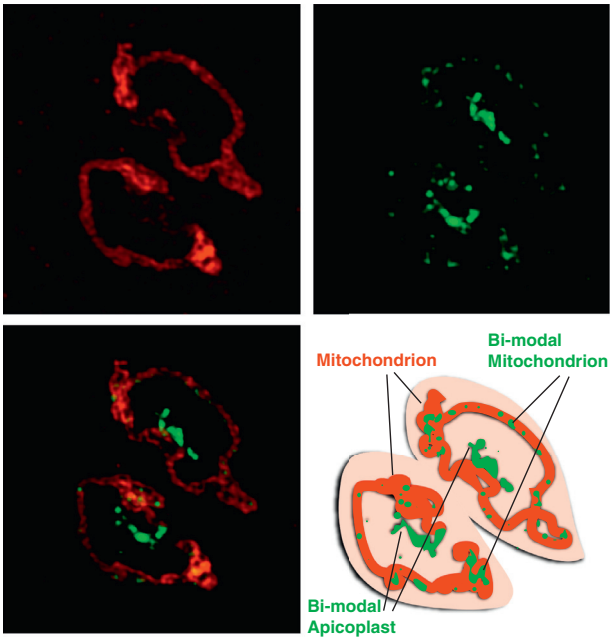
Mammalian cells have varying numbers of mitochondria that divide or fuse based on changing cellular needs, whereas Apicomplexa possess a single mitochondrion whose biogenesis coordinates with the cell-cycle [2]. Transfer of mitochondrial genes to the nucleus has occurred in all eukaryotes, allowing nuclear control over mitochondrial functions (Figure 2). The resulting loss of mitochondrial DNA-encoded genes is extreme in Apicomplexa and dinoflagellates, whose mitochondrial genomes encode only three proteins [6,7]. The organellar proteome is largely imported from the cytosol, presumably through the **t**ranslocons of the **o**uter and **i**nner **m**itochondrial membranes (TOM and TIM [8]) as with other eukaryotes. Translation within the Apicomplexa mitochondrion, however, is highly divergent. Extremely fragmented ribosomal RNA genes encode products that need to be assembled into functional ribosomes [9]. No tRNAs are encoded in the mitochondrial genome [6], and no tRNA amino acyl synthetases are targeted to the organelle [10,11], rendering mitochondrial translation dependent on a flow of charged tRNAs from the cytosol, an extremely unusual process.

Mitochondria are essential for both *Toxoplasma* and *Plasmodium* spp., being the synthetic site for a number of metabolites (reviewed in [5,8]). However, recent data suggest that they differ in the composition and importance of their oxidative phosphorylation pathways.

## Oxidative phosphorylation and TCA cycle

Oxidative phosphorylation is a canonical function of eukaryotic mitochondria. Tricarboxylic acid (TCA) cycle reactions are the chief source of electrons that feed the mitochondrial electron transport chain (mtETC), generating a proton gradient used for ATP synthesis by the ATP synthase complex (Figure 3).

**Figure 1**




Fluorescence image of the mitochondrion and the apicoplast of *Toxoplasma gondii*. The staining of a mitochondrial protein (TGME49_215430, [11], red) that localizes to the organelle periphery (Sheiner, unpublished data) together with a bimodally targeted mitochondrial luminal and apicoplast protein (TGME49_283830, Sheiner, unpublished data, green) shows the tight proximity between the two organelles. The co-staining of the mitochondria demonstrates the difference in morphology between the luminal and peripheral compartments. TGME49_283830 (green) represents one of many examples of bimodal targeting between the two organelles. The scheme on the right depicts the outline of the two *Toxoplasma* tachyzoites. Bar is 1 μm.

Genomic sequencing of *Toxoplasma gondii* and *Plasmodium* spp. revealed genes encoding all TCA cycle enzymes, most mtETC components and most ATP synthase complex subunits. Selective inhibition of mtETC leads to parasite demise, establishing the essential nature of these reactions. In *Toxoplasma*, mtETC inhibition affects ATP synthesis [12], suggesting the presence of oxidative phosphorylation. However, in *Plasmodium* erythrocytic stages, mtETC contribution to the ATP pool seems minor [13]. Instead, mtETC appears essential for pyrimidine biosynthesis by re-oxidation of ubiquinol, needed for the mitochondrially located dihydroorotate dehydrogenase (DHODH) [14]. While these results suggest that oxidative phosphorylation is not essential for *Plasmodium* erythrocytic stages, ATP synthase subunits are resistant to genetic disruption in these stages [15].

The *Toxoplasma* TCA cycle utilizes glucose and glutamine, as judged by stable isotope labeling and metabolomic analysis, and a GABA shunt was noted for entry of glutamine into the cycle [4••] (Figure 3). The source of acetyl-CoA for priming the cycle is unclear, since the only known pyruvate dehydrogenase complex resides in the apicoplast [16–18]. Branched-chain keto acid metabolism has been proposed as an alternative source [5].

In *Plasmodium*, stable isotope labeling and metabolomic analyses initially suggested that TCA metabolism involved a branched architecture bifurcating from 2-oxoglutarate [19]. However, subsequent investigations revealed that products of the seemingly reductive branch originate from uninfected erythrocytes [20] and the initial report was retracted [21]. Highly enriched parasite-infected cells show only conventional oxidative reactions, with 2-oxoglutarate as the entry point (Ke *et al*., unpublished data). Unlike *Toxoplasma*, glutamine rather than glucose is the major carbon source for the TCA cycle in *Plasmodium* erythrocytic stages [20]. Genetic disruptions of six TCA cycle enzymes suggest that the TCA cycle is not essential for *Plasmodium* erythrocytic-stage or sexual-stage development, but is necessary for mosquito stage development (Ke *et al*., unpublished data). Similarly, in *Plasmodium berghei*, where the mtETC components NADH dehydrogenase [22] and succinate dehydrogenase [23] are dispensable for erythrocytic stages, they are essential for mosquito oocyst formation.

## Mitochondrial involvement in cell-death and differentiation

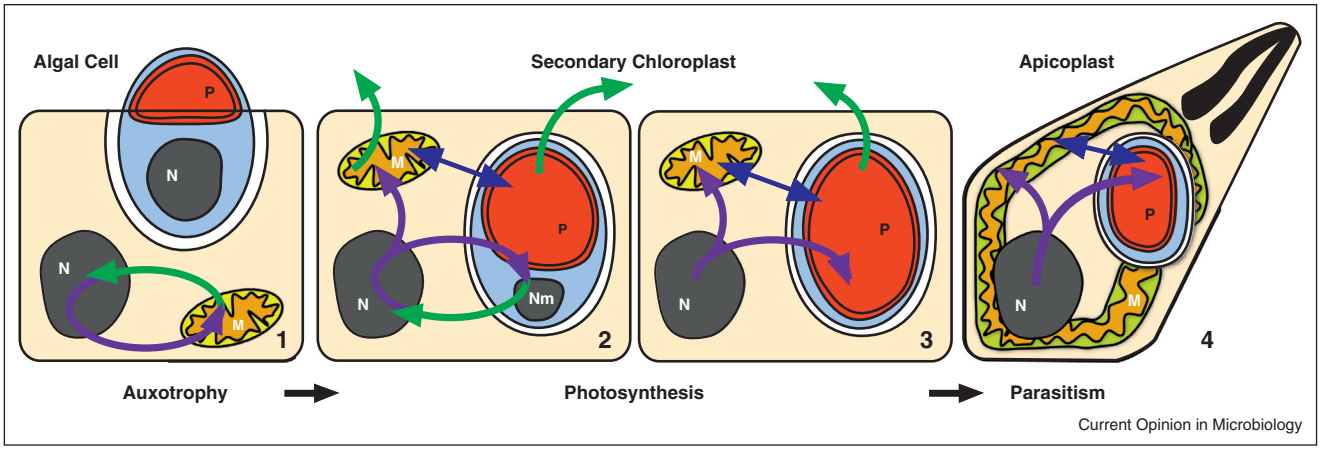
Recent studies link mitochondrial dynamics and autophagy in *Toxoplasma* [24•,25,26]. Mitochondrial fragmentation was observed in response to both autophagy inhibition [24•] and activation [25,26], creating contradictory models where autophagy either controls mitochondrial homeostasis or induces cell death. Interestingly, autophagy-mediating components associate with the apicoplast [27], and overexpression of one of them, TgATG4, results in mitochondrion and apicoplast morphological defects [27], supporting the first model. However, inhibition of autophagy led to prolonged parasite survival under monensin treatment [26] supporting the second model.

The involvement of a mitochondrial DnaK tetratricopeptide repeat protein in tachyzoite-to-bradyzoite differentiation was recently proposed, joining several previous studies demonstrating a correlation between reduced mitochondrial activity and stage differentiation [28]. The mechanism remains unknown.

## Haem biosynthesis, a mitochondrion/apicoplast collaboration

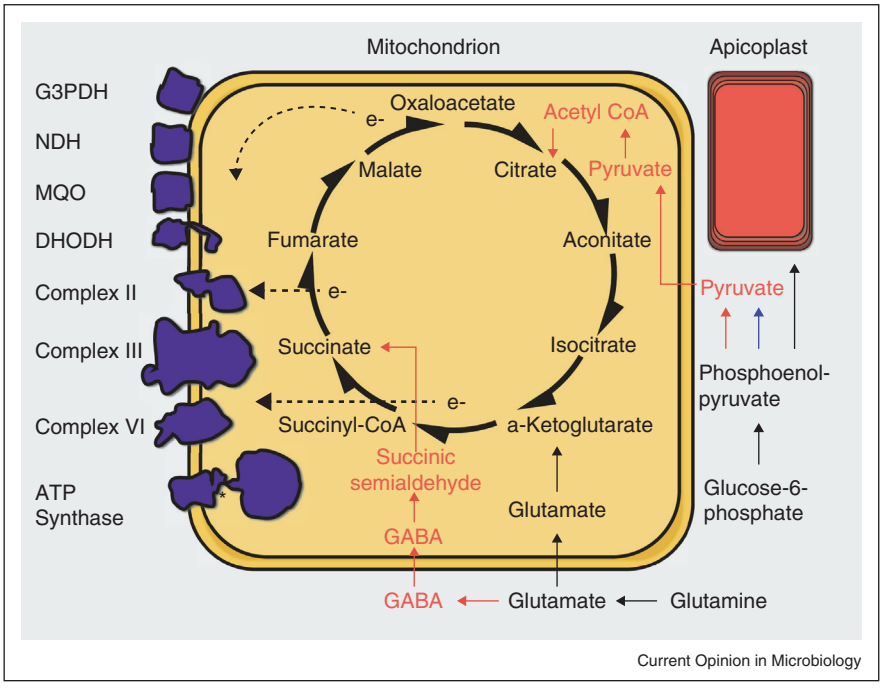
The genomes of *Plasmodium* and *Toxoplasma* encode the complete set of haem synthesis genes [29]. Like most non-photosynthetic organisms, the pathway starts with mitochondrial conversion of glycine into δ-aminolaevulinic

**Figure 2**



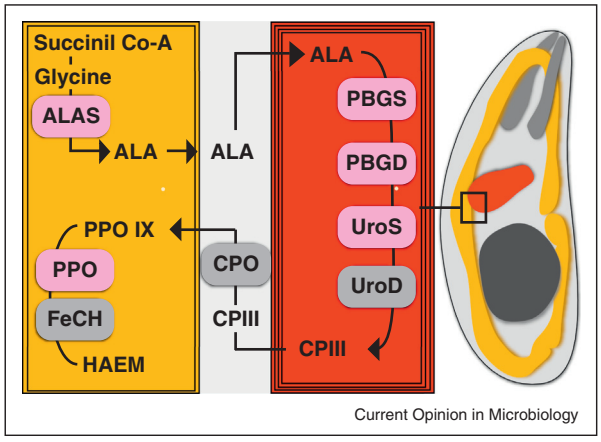

Schematic outline of the acquisition and evolution of the mitochondrion and the apicoplast of Apicomplexa. (1) Development of a protein import system (purple arrows show flow of proteins from their genomic place of encoding to their subcellular localization) was an important event in the evolution of a mitochondrion in the ancestor of all eukaryotes. This was accompanied by extensive gene transfer to the nuclear genome (green arrows indicate the transfer of genes to another genome or their complete loss). An algal cell (light blue) carrying a plastid (red) then began an endosymbiotic relationship with a protist host. (2) Again protein import systems were established supporting the extensive gene transfer to the nuclear genome and allowing nuclear control over a newly enslaved organelle. A stable collaboration between the two symbionts (blue arrow) drove the loss of some redundant genes (green arrows). (3) A subsequent loss of photosynthesis (green arrow) affected the distribution of tasks, such as haem synthesis, between the two organelles (blue arrow). (4) Finally the two symbionts now present in apicomplexan parasites are synchronized in their biogenesis and are tightly associated, although the biological role of this association remains unclear. (M) mitochondrion, (P) plastid, (N) nucleus, (Nm) nucleomorph.

**Figure 3**




TCA and mtETC in *Plasmodium* erythrocytic stages and in *T. gondii* tachyzoites, Pyruvate from glycolysis, glutamate and gamma aminobutyric acid (GABA) from glutamine metabolism all serve as major starting points for the *Plasmodium* (thin black arrows, and a thin blue arrow representing a putative pathway) and *Toxoplasma* (thin black and red arrows) TCA cycles. Electrons from the oxidative steps in the cycle are donated to the mtETC (represented as broken arrows). Components of the mtETC are shown in purple with their names noted on the left. The asterisk notes that not all the subunits of the Apicomplexa ATP-synthase are identifiable in their genomes.

**Figure 4**




A chimerical haem biosynthesis pathway in Apicomplexa. The acquisition of photosynthesis and then its subsequent loss resulted in shifts as to which compartment was the main user of tetrapyrroles in the cell, and with it the location of principal responsibility for synthesis. The resulting pathway is distributed between the mitochondrion (orange), cytosol (gray) and apicoplast (red). Similarly, the enzymes involved are of different origins within the original endosymbiont [31•]: either the red-algal plastid (pink) or cytoplasm (gray).

acid [30]. However, the cellular localization and phylogenetic origin of the downstream enzymes tell a tale of evolutionary shuffling and rejigging. The next four steps, executed by HemB/C/D/E respectively, take place in the plastid. While HemB/C/D are of plastid origin, HemE originates from the ancestral eukaryotic host cell, an ancestry not reflected by its current place of action [31•]. The subsequent steps are executed by a cytosolic HemF, and then by mitochondrial HemY and HemH. Interestingly, the mitochondrial HemY derives from the red-algal ancestor of the apicoplast [32], again a conflict between ancestry and current location. Thus, the pathway wends its way through three compartments, employing enzymes of various ancestral pathways, only to wind up back in the mitochondrial start point (Figure 4). This curious hybrid pathway likely reflects the shifts in the main sites of use for tetrapyroles following the acquisition and subsequent loss of photosynthesis [3].

## The apicoplast

A common ancestor of Apicomplexa and dinoflagellates engulfed a red alga, which underwent reduction to become a secondary plastid (Figure 2). Most dinoflagellates maintained a photosynthetic plastid, unlike the apicomplexan plastid — the apicoplast — which lost photosynthesis. The apicoplast now supports three essential metabolic functions: the synthesis of haem (above), type II fatty acids, and isoprenoid precursors.

## Type II fatty acid synthesis (FASII)

Fatty acids are a core component of cellular membranes and of essential prosthetic groups [33]. *De novo* fatty acid synthesis occurs either via fatty acid synthesis pathway I (FASI), typically found in animals and fungi and executed by a cytosolic multi-domain polypeptide, or via FASII, which depends on several individual enzymes and is more common in prokaryotes and plastids.

Both the *Toxoplasma* and *Plasmodium* genomes encode complete sets of FASII enzymes [34], and several kinetic, structural and pharmacological studies support the roles of the corresponding proteins in FASII (reviewed in [30,35]). However, FASII was apparently lost by some Apicomplexa [36], and its importance for parasite survival differs between genera and life stages. Genetic evidence indicates that FASII is essential for the growth of *Toxoplasma* tachyzoites and *Plasmodium* liver stages but not erythrocytic or mosquito stages [37,38]. This suggests that the importance of FASII depends on the host cell or tissue environment. A recent study using lipidomics and uracyl incorporation in *Plasmodium* asexual stages suggests that the biogenesis of the apicoplast, and potentially other organelles, depend on salvaged precursors rather than *de novo* fatty acid synthesis in these stages [39••].

The loss of lipoylation of plastid pyruvate dehydrogenase observed with both pharmacological [16] and genetic [40] disruption of *Toxoplasma* FASII had suggested that FASII supplies only specialized apicoplast lipids. However, a recent study combining metabolomic and genetic analyses indicated that most (60–80%) myristic and palmitic acids in *Toxoplasma* originate from FASII activity [41•], making the apicoplast a significant source of cellular fatty acids. The remaining 20–40% are presumably derived from other sources, perhaps including the homolog of the multifunctional FASI enzyme found in the *Toxoplasma* genome, a potential remnant of its pre-photosynthetic ancestor. There is also clear evidence for lipid salvage from the host [39••,42], and it appears that the contributions of *de novo* synthesis and salvage vary depending on circumstances. This flexibility perhaps facilitates the transition of parasites through different types of host cell during their complex life cycle.

## Isoprenoid precursor biosynthesis

Isoprenoids are derivates of IPP or of its isomer dimethylallyl pyrophosphate (DMAPP). Apicomplexans possess the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway for IPP synthesis [29,43], which is found mainly in eubacteria and plastids, and lack the alternative mevalonate pathway found in the cytosols of plant, animal and fungal cells.

*Plasmodium* spp. are sensitive to fosmidomycin [43], an inhibitor with two potential targets in the DOXP pathway [44]. Yeh and DeRisi showed that IPP can negate the effect of fosmidomycin, reinforcing the drug's specificity

[45••]. Moreover, plastid-less *Plasmodium falciparum* blood stages can be propagated in the presence of exogenous IPP, implicating the DOXP pathway as the only essential apicoplast function in *Plasmodium* erythrocytic stages [45••]. Nair and coworkers used genetic approaches to confirm that the DOXP pathway is essential in *Toxoplasma*, although fosmidomycin showed a little or no effect on tachyzoite growth [46]. Expressing a bacterial fosmidomycin transporter rendered *Toxoplasma* fully susceptible to fosmidomycin, suggesting that drug accessibility dictates sensitivity in this case [46]. In an independent study, Baumeister and coworkers reached a similar conclusion but suggest the barrier to drug entry is the host-cell rather than the parasite membranes [47].

The end uses of parasite-synthesized IPP are becoming clearer. Potential products include membrane anchors for dolichols in the ER glycosylation machinery and for ubiquinone in the mtETC. IPPs are also precursors of the prenyl tails of a range of C-terminally prenylated proteins such as Rabs [48], which are common in both *Toxoplasma* and *Plasmodium*.

## Concluding remarks

The endosymbiotic organelles of Apicomplexa are crucial for parasite survival in different host settings during their complex life cycle. Studies combining metabolomics and genetic approaches have exposed interesting differences between *Plasmodium* and *Toxoplasma* in the roles of certain pathways. While genetic studies suggest that the TCA cycle is dispensable for *Plasmodium* erythrocytic stages, pharmacological evidence supports an essential role in *Toxoplasma* tachyzoite growth [4••].

Similarly, the apicoplast FASII pathway is essential in *Toxoplasma* tachyzoites but dispensable in *Plasmodium* erythrocytic stages, where IPP precursor synthesis is the only essential function.

These differences may reflect the specialist versus generalist strategies adopted by *Plasmodium* and *Toxoplasma*. Malaria parasites appear to rely less on organelle metabolism in erythrocyte stages. Conversely, *Toxoplasma* tachyzoites, which can parasitize a large range of host cells, salvage less from their host and are more dependent on self production.

Another explanation might be related to the different properties of erythrocytes and nucleated cells. This is supported by the importance of the FASII pathway in *Plasmodium* liver stages and the dependence of mosquito stages on an active TCA cycle — both findings are similar to those in *Toxoplasma* tachyzoites.

In contrast to our growing understanding of the apicoplast and mitochondrion metabolic roles, their biogenesis is currently understudied. Insights into apicoplast protein import [49–52] and division [53,54] are beginning to accumulate, pioneering this important aspect of organellar biology. Unbiased strategies are being developed aimed at enlarging the repertoire of known apicoplast proteins [55•] and isolating apicoplast enriched fractions [39••]. A lipidomics study performed with isolated *Plasmodium* asexual stage apicoplasts revealed that the majority of lipids incorporated in the apicoplast membranes are likely of host rather than algal origin [39••]. The relative contribution of *de novo* synthesis and salvage pathways to the biogenesis of the apicoplast in *Toxoplasma* is yet to be established.

Apicomplexan mitochondrial biogenesis is an even more neglected area of research. Its tight association with the apicoplast has impaired the attempts to address this question. The establishment of biogenesis mutants for both organelles [49–54,55•] (Sheiner, unpublished data) paves the way to develop strategies based on breaking their association and isolating each organelle for its separate analysis.

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- •• of outstanding interest

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