Trends in Parasitology

CellPress

Review

# Apicomplexan Energy Metabolism: Carbon Source Promiscuity and the Quiescence Hyperbole

Damien Jacot,[1] Ross F. Waller,[2] Dominique Soldati-Favre,[1] Dougal A. MacPherson,[3] and James I. MacRae[4,*]

**The nature of energy metabolism in apicomplexan parasites has been closely investigated in the recent years. Studies in *Plasmodium* spp. and *Toxoplasma gondii* in particular have revealed that these parasites are able to employ enzymes in non-traditional ways, while utilizing multiple anaplerotic routes into a canonical tricarboxylic acid (TCA) cycle to satisfy their energy requirements. Importantly, some life stages of these parasites previously considered to be metabolically quiescent are, in fact, active and able to adapt their carbon source utilization to survive. We compare energy metabolism across the life cycle of malaria parasites and consider how this varies in other apicomplexans and related organisms, while discussing how this can be exploited for therapeutic intervention in these diseases.**

## Apicomplexan Mitochondria – A Missing Link and an Absent Cycle

The phylum Apicomplexa comprises a vast group of unicellular parasites that are the causative agents for some of the world's most important infectious diseases. Distinctive features of Apicomplexa include a specialized apical invasion machinery, termed the apical complex, and a relict plastid derived from a red algal secondary endosymbiont known as the apicoplast [1,2]. The human parasites *Plasmodium* spp. and *Toxoplasma gondii* represent the best-studied, and among the most medically-relevant, members of this large phylum. Collectively, these obligate intracellular parasites impose an enormous burden on human health [e.g., *Plasmodium* (malaria), *Toxoplasma* (toxoplasmosis), *Cryptosporidinum* (cryptosporidiosis)], food production and economy (e.g., *Babesia*, *Theileria*, *Eimeria*), and a largely unexplored impact on wild animal populations. A better appreciation of their metabolism, including adaptive capabilities, is of primary importance to understanding the life cycles of these parasites and their ability to thrive in secluded intracellular environments. Recent advances in the field of metabolomics have revealed important parasite-specific nuances in apicomplexan energy generation, shedding light on the biochemistry behind the success of these organisms.

In many eukaryotes, the mitochondrion is the power station of the cell that allows complete oxidation of carbohydrates, lipids, and amino acids via the tricarboxylic acid (TCA) cycle, leading to efficient ATP generation through the electron transport chain (ETC). In Apicomplexa, a single tubular mitochondrial network also hosts part of heme biosynthesis, iron–sulphur (Fe–S) cluster assembly, and lipoic acid salvage, and also participates in the synthesis of many metabolic intermediates including pyrimidines (as reviewed elsewhere [3,4]). However, the role of the

### Trends

Apicomplexans adjust their carbon source usage depending on their developmental stage. *Plasmodium* asexual stages rely on aerobic fermentative glycolysis, switching to a greater flux through the TCA cycle upon sexual differentiation. In *Toxoplasma gondii* asexual 'tachyzoites', glucose and glutamine feed into an active TCA.

Apicomplexans use enzymes in non-traditional ways to meet their energy needs. Branched-chain ketoacid dehydrogenase (BCKDH) acts as the absent mitochondrial pyruvate dehydrogenase (PDH); other enzymes have been relocated, operate in a non-canonical direction, or have been replaced entirely.

Enzymes of mitochondrial energy metabolism show potential as targets to intervene in the transmission and treatment of the diseases caused by apicomplexan parasites.

[1]Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[2]Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, UK
[3]15 Minute Drawings, Haughton Road, Oakleigh South, Victoria 3167, Australia
[4]The Francis Crick Institute, The Ridgeway, Mill Hill, London NW7 1AA, UK

*Correspondence: james.macrae@crick.ac.uk (J.I. MacRae).

apicomplexan mitochondrion in energy generation has remained unclear, given the glucose-replete intracellular nature of the environmental niches of these parasites and the apparent reduction of mitochondrial metabolic pathways [4–7]. For example, apicomplexan mitochondria lack 'type I' NADH dehydrogenase (NDH, complex I of the ETC) and many apparently lack the enzymes and transporters required for fatty acid β-oxidation [8,9]. Furthermore, the pyruvate dehydrogenase complex (PDH) responsible for conversion of pyruvate into acetyl-CoA – an obligate fuel for the TCA cycle – is only present in the apicoplast [10]. Consequently, there was no obvious entry point to allow catalysis of glycolytic pyruvate by the TCA cycle [10–14]. Despite this, there is overwhelming evidence that apicomplexans rely on a functional and canonical TCA cycle (as reviewed [4,5,15]), and apicomplexan genome annotations indicate the presence of all enzymes of the TCA cycle. Only one clade of apicomplexans, *Cryptosporidium* spp., show evidence of outright loss of the TCA cycle, but these taxa retain only a highly reduced mitochondrion, the mitosome, and have also lost their apicoplast [5,8,16].

This review highlights how metabolomics technologies coupled to genetic manipulation have helped in unraveling apicomplexan parasite plasticity in carbon source utilization through different life stages, revealing a complex and sometimes counter-intuitive pattern of metabolic gains, losses, and reassignments. These changes have presumably been tailored to allow these parasites to thrive within their various host niches, but may constitute some much-needed Achilles' heels in the fight against these devastating diseases.

## *Plasmodium falciparum* and the Glycolytic Deceit

Glycolysis has long appeared to be the main source of ATP and NAD(P)H in the erythrocytic stages of the malaria parasites, with oxidative phosphorylation thought to only occur in the mosquito vector stages [5,17–21]. Indeed, glucose uptake in *P. falciparum*-infected red blood cells (RBCs) has been observed to increase 75–100-fold compared to uninfected RBC [22–25]. Up to 93% of glucose is converted directly to lactate in asexual stages [26] and is ultimately excreted into the surrounding host cell. Perturbation of glucose uptake is detrimental to parasite growth [27,28], suggesting that glycolysis is an important part of the parasite's strategy for rapid proliferation. In a manner analogous to the Warburg effect observed in highly-proliferative cancer lines and other rapidly-growing cells (e.g., yeast and bloodstream *Trypanosoma* spp.), *Plasmodium* (in erythrocytic stages) has opted for fast generation of ATP through substrate-level phosphorylation and secretion of lactate as an end-product (aerobic fermentative glycolysis), as opposed to the 'slow but efficient' mitochondrial oxidative phosphorylation and complete oxidation [29]. Reasons for this dependence on glycolytic fermentation remain unclear – after all, blood stages of *Plasmodium* certainly have access to oxygen – but it may be a way of avoiding excessive reactive oxygen species (ROS) production in an environment already under considerable stress due to hemoglobin breakdown [30,31]. In any case, reliance on glycolysis led to the assumption that mitochondria might be substantially obsolete in blood-stage parasites.

Despite the strong dependence on glycolysis, it is now clear that blood stages of *Plasmodium* spp. do require mitochondrial metabolism because they are susceptible to drugs targeting the ETC, such as atovaquone, that inhibits complex III [32–37]. Evidently this is not for ATP synthesis because inhibition of the ETC has little effect on cellular ATP levels [38–40], and deletion of the functional β-subunit of ATP synthase can be tolerated in blood stages of *P. berghei* [31]. Heme biosynthesis, which is reliant on TCA-derived succinyl-CoA, is dispensable in asexual blood stages [41,42], and thus dissipation of reducing power from TCA activity for this pathway is not required. Perhaps, then, only re-oxidation of ubiquinol, which is necessary to support the function of the ubiquinone-dependent dihydroorotate dehydrogenase (DHODH) for pyrimidine synthesis, requires ETC activity in blood stages. Provided that ubiquinone turnover is maintained, which minimally requires complex III, the initial two complexes of