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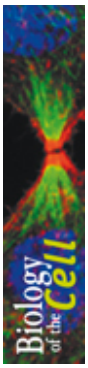
Review



Versatility in the acquisition of energy and carbon sources by the Apicomplexa

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Members of the phylum Apicomplexa are motile and rapidly dividing intracellular parasites, able to occupy a large spectrum of niches by infecting diverse hosts and invading various cell types. As obligate intracellular parasites, most apicomplexans only survive for a short period extracellularly, and, during this time, have a high energy demand to power gliding motility and invasion into new host cells. Similarly, these fast-replicating intracellular parasites are critically dependent on host-cell nutrients as energy and carbon sources, noticeably for the extensive membrane biogenesis imposed during growth and division. To access host-cell metabolites, the apicomplexans *Toxoplasma gondii* and *Plasmodium falciparum* have evolved strategies that exquisitely reflect adaptation to their respective niches. In the present review, we summarize and compare some recent findings regarding the energetic metabolism and carbon sources used by these two genetically tractable apicomplexans during host-cell invasion and intracellular growth and replication.

Introduction

The phylum Apicomplexa contains thousands of species, including several important human and animal pathogens. *Toxoplasma gondii* infects almost all warm-blooded animals and is opportunistic in humans, causing encephalitis or systemic infections in immunocompromised patients, in addition to severe congenital birth defects when women are primarily infected during pregnancy (Weiss and Dubey, 2009). *Plasmodium falciparum*, the causative agent of the most deadly form of malaria, is responsible for up to two million deaths per year (Snow et al., 2005; Guinovart et al., 2006).

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Key words: Apicomplexa, glucose metabolism, glutaminolysis, *Plasmodium falciparum*, *Toxoplasma gondii*, tricarboxylic acid cycle (TCA cycle).

Abbreviations used: ACL, ATP citrate lyase; ACP, acyl-carrier protein; APT, apicoplast phosphate translocator; AQP, aquaglyceroporin; BCAA, branched-chain amino acid; BCKDH, branched-chain ketoacid dehydrogenase; ER, endoplasmic reticulum; FA, fatty acid; FASI, type I FA synthetase; FASII, type II FA biosynthesis; GK, glycerol kinase; INDY, I'm not dead yet; MCC, methylcitrate cycle; NPP, new permeation pathway; OAA, oxaloacetate; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PfHT, *P. falciparum* hexose transporter; PV, parasitophorous vacuole; PVM, parasitophorous vacuole membrane; RBC, red blood cell; iRBC, infected RBC; TCA, tricarboxylic acid; TgGT1, *T. gondii* glucose transporter 1.

Inside the host cells, *T. gondii* and *P. falciparum* develop inside a unique PV (parasitophorous vacuole) created upon active parasite entry and produced mainly by the invagination of the host cell membrane. Development within such a non-fusogenic PV confers the advantage of creating a safe niche that protects the intracellular parasite from lysosomal degradation and minimizes exposure to the immune system. Consequently, the parasite is separated from the nutrients of the host by a series of physical barriers, including the host-cell plasma membrane, the PVM (parasitophorous vacuole membrane) and the pellicle of the parasite.

Following a single passage in hepatocytes, *P. falciparum* establishes a cyclic infection in differentiated enucleated human RBCs (red blood cells) and uses glucose from the blood as an unlimited source of energy for its survival and replication. In contrast, *T. gondii* grows within virtually any biosynthetically active cell type and, in consequence, competes directly with host cells for metabolites, such as nucleotides, glucose, lipids and amino acids. The fast replication rate of these parasites results in a high demand for energy and carbon sources, notably to sustain membrane biogenesis. To accommodate

their needs, the apicomplexans have evolved distinct strategies, including some ingenious metabolic adaptations to their niche and lifestyles (Seeber et al., 2008). The present review summarizes recent findings and discusses future prospects regarding the energy and carbon sources exploited by *T. gondii* and *P. falciparum*.

Parasite strategies to access host nutrients

P. falciparum and *T. gondii* are secluded within a PVM during the whole intracellular replication cycle. This compartment serves as a shelter against host-cell defence mechanisms, but necessitates the development of strategies to gain access to nutrients. Ultrastructurally, these parasites are separated from the extracellular milieu by three systems of membranes: the host-cell plasma membrane, the PVM and the parasite plasma membrane.

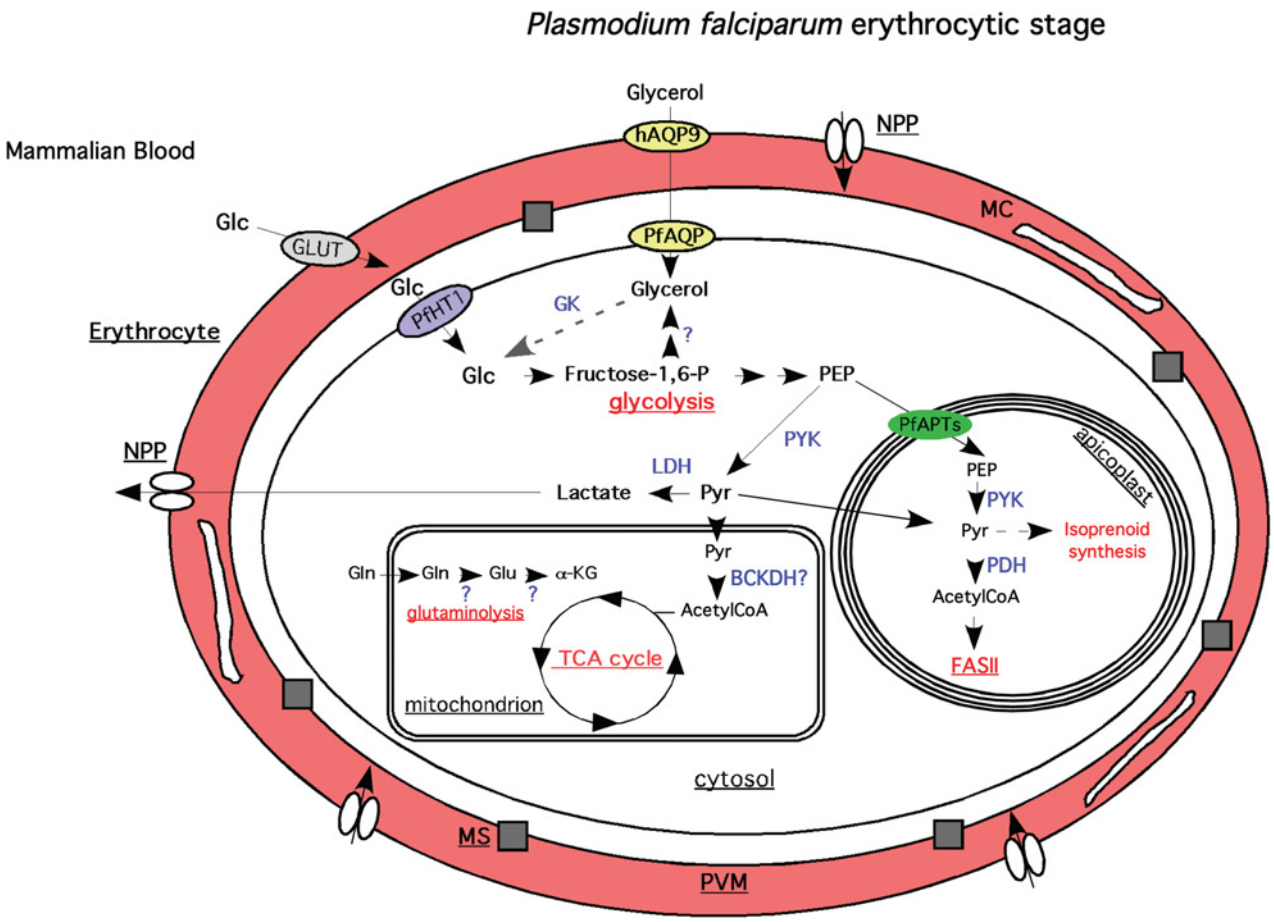
Despite displaying reduced metabolic activities and limited transport capacities, RBCs serve as a suitable niche for the malaria parasites. First of all, *P. falciparum* relies on GLUT-1 (glucose transporter type 1), the glucose transporter expressed at the surface of human RBCs (Kirk et al., 1996). It has been known for a long time that *P. falciparum* actively modifies the permeability of the iRBC (infected RBC) membrane to optimize the uptake of nutrients (Kutner et al., 1983). The export of parasite proteins from the RBC surface dramatically changes the properties of the plasma membrane and, notably, contributes to the induction of the so-called NPP (new permeation pathway) (Figure 1). The activation of the NPP results in the augmented transport in iRBCs of ions, isoleucine, glutamine, monosaccharide sugars, peptides, nucleosides and pantothenate, which are essential for parasite growth (reviewed by Kirk, 2001; Baumeister et al., 2006). The nature of the transporters responsible for this enhanced import of a wide range of low-molecular-mass molecules has not been identified to date, and their properties are a matter of controversy (Staines et al., 2007). The NPP has also been proposed to contribute to the export of waste products, such as lactate from glycolysis and amino acids from haemoglobin degradation, to the extracellular milieu (Saliba and Kirk, 2001; Martin and Kirk, 2007). In contrast, *T. gondii* develops in nucleated cells and has not been reported to modify membrane permeability significantly, although such a phenomenon might not have been sufficiently investigated.

Immediately following its formation during invasion, the PVM of *T. gondii* and *P. falciparum* is subjected to considerable modifications. Host-cell mitochondria and ER (endoplasmic reticulum) are recruited at the PVM of *T. gondii*, presumably to allow the scavenging of host lipids, possibly including cholesterol (Sinai et al., 1997; Charron and Sibley, 2002) and glycan intermediates (Garenaux et al., 2008). At the liver stage of *Plasmodium*, the PVM was observed to associate with the host ER, but apparently not with mitochondria (Bano et al., 2007). Moreover, *T. gondii* was also described to gain access to a diverse range of low-molecular-mass components (metals, sulfate, lipids, amino acids and sugars) through an unusual recruitment of the host endolysosomal system into the PV (Coppens et al., 2006).

In addition to these exchanges made possible by the intimate association with the host organelle, the PVM of *T. gondii* tachyzoites acts as a molecular sieve, allowing the free exchange of molecules smaller than 1.4 kDa (i.e. sugars, nucleotides and amino acids) between the host cytosol and the PV space (Schwab et al., 1994) (Figure 2A). This free permeability of the PVM plays a critical role in the context of the multiple parasite auxotrophies (arginine and tryptophan). Similarly, the PVM enclosing *P. falciparum* in iRBCs also allows the free passage by diffusion through channels of low-molecular-mass ions, nutrients and metabolic waste (Kirk, 2001). The PVM formed by the intrahepatic parasite *Plasmodium berghei* was shown to function as a molecular sieve to solutes smaller than 855 Da (Bano et al., 2007). The nature and identity of the pore inserted by the apicomplexans in their PVM have not been elucidated to date.

In addition to host-cell remodelling and free diffusion through the PVM, the apicomplexans have developed strategies to overcome the third barrier, the parasite plasma membrane. Except for the unusual uptake of haemoglobin into the food vacuole of the malaria parasites through the cytostome (Elliott et al., 2008; Abu Bakar et al., 2010), *T. gondii* and possibly other apicomplexans show no evidence for substantial endocytotic activity. Consequently, they are anticipated to express a large variety of transporters to ensure specific uptake of metabolites from the host cytosol. More than 2.5 % of the *P. falciparum*

Figure 1 | Metabolic pathways used by *Plasmodium falciparum* intra-erythrocytic stages to produce energy
P. falciparum intracellular stages are separated from the host cytosol by a PVM that acts a molecular sieve (MS) to facilitate uptake of small nutrients. *P. falciparum* is able to modify the membrane of the erythrocyte by the NPP to allow the uptake of ions and numerous metabolites from the blood. The parasite plasma membrane is also modified by specific transporters to acquire the carbon and energy sources needed. The glycerol can be used via gluconeogenesis to produce glycolysis intermediates and glucose. Glutamine can also be a source of TCA intermediates. Glc, glucose; GLUT, glucose transporter; α-KG, α-ketoglutarate; MC, Maurer's cleft; PYK, pyruvate kinase; Pyr, pyruvate.



genome is dedicated to genes coding for transporters and channels; however, the subcellular localization and physiological role of only a small proportion of them are known (Martin et al., 2009).

Energy metabolism in *P. falciparum* and *T. gondii*

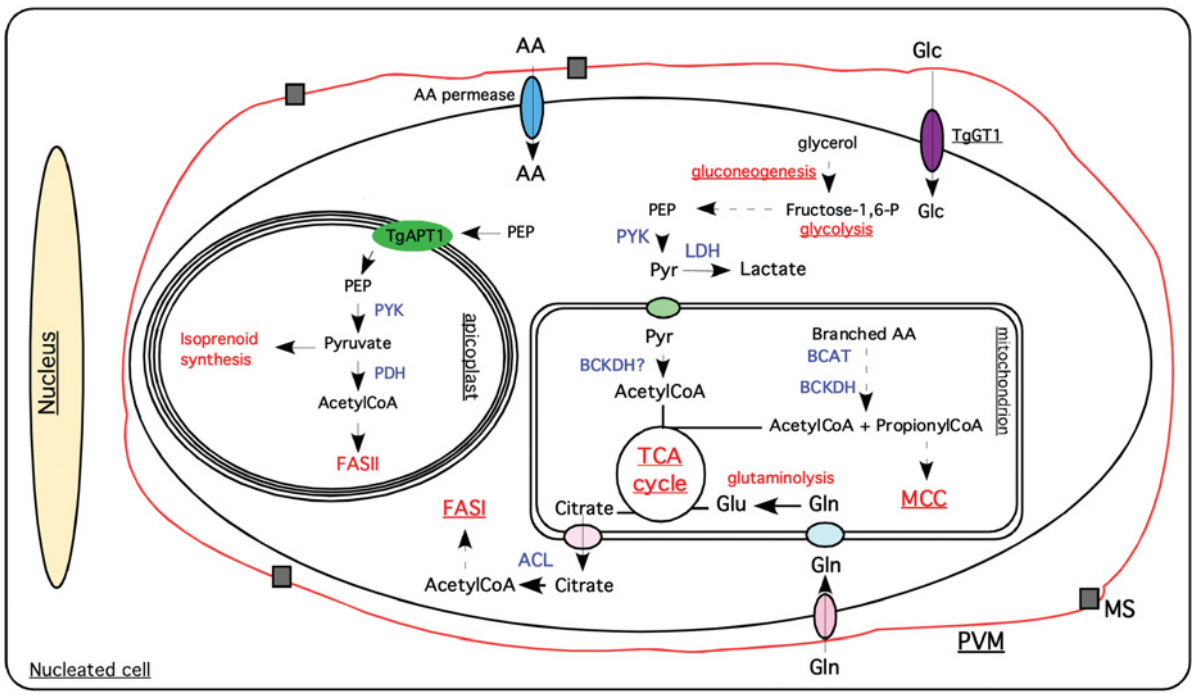
Our understanding of the energy metabolism in apicomplexans is fragmented, owing to the intricate interconnections with the host-cell metabolism and the radical metabolic changes occurring during stage differentiation throughout the parasite life cycle. It is wise not to make general statements because of the considerable diversity of the stages and the experimental limitations that fail to reflect exactly the *in vivo* conditions or even hamper the cultivation of some stages.

Plasmodium species and *T. gondii* possess the complete sets of genes coding for the TCA (tricarboxylic acid) cycle and glycolytic enzymes (Figures 1 and 2); however, the importance of a functional TCA cycle and contribution of oxidative phosphorylation in energy production is uncertain. Evidence for a functional respiratory chain and oxidative

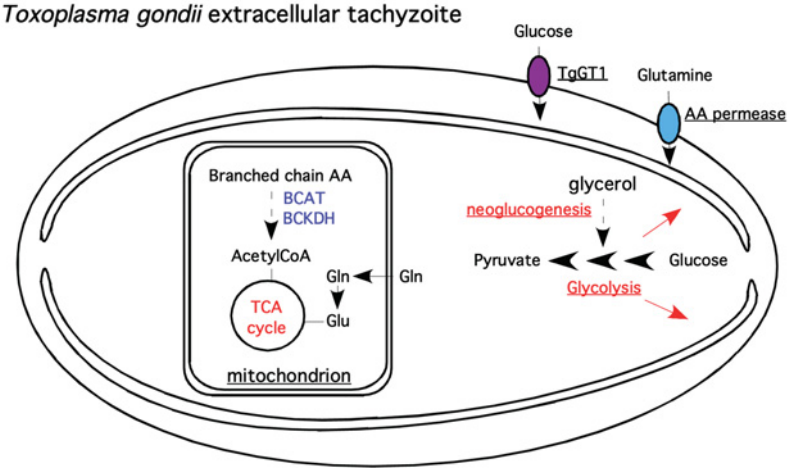
Figure 2 | Representation of the interconnected metabolic pathways generating energy and carbon sources during intracellular growth of *T. gondii* (A) and in extracellular parasites (B)

T. gondii intracellular stages are separated from the host cytosol by a PVM that acts a molecular sieve (MS) to facilitate uptake of small nutrients. These parasites have also developed specific transporters on their plasma membrane to acquire carbon sources they need. They use glucose via glycolysis to generate pyruvate, which is then used by the PDH complex restricted to the apicoplast, allowing the generation of acetyl-CoA used for FASII to generate new membranes. The hypothetical pathways leading to acetyl-CoA synthesis in the mitochondria to fulfil the TCA-cycle energy production and citrate synthesis necessary for cytosolic FASI are presented for both parasites. Glutamine is also an important carbon source, generating α-ketoglutarate to fulfil the TCA cycle and produce energy. AA, amino acids; BCAT, branched-chain amino acid transferase; Glc, glucose; LDH, lactate dehydrogenase; PYK, pyruvate kinase; Pyr, pyruvate.

A. *Toxoplasma gondii* intracellular tachyzoite



B. *Toxoplasma gondii* extracellular tachyzoite



phosphorylation were reported in *T. gondii* (Vercesi et al., 1998) and in *P. yoelii yoelii* (Uyemura et al., 2004). The gene coding for succinyl-CoA synthase was disrupted in *T. gondii,* and the resulting mutant showed only a 30 % reduction in growth rate that could be restored by supplying it with exogenous succinate (Fleige et al., 2008).

In addition to the ten enzymes constituting the glycolytic pathway in the cytosol, *T. gondii* also expresses glycolytic enzymes targeted to the apicoplast (Figure 2A), where they might contribute to the local production of ATP (Fleige et al., 2007). In contrast, the source of energy for the apicoplast of the malaria parasite is currently unknown. Interestingly, the enzymes involved in gluconeogenesis are present in *T. gondii*, but lacking in *Plasmodium* spp., a feature that reflects the differential accessibility to glucose in the niches occupied by these two parasites.

The malaria-parasite intraerythrocytic stages have been considered for a long time to rely exclusively on incomplete oxidation of glucose, leading to the accumulation and secretion of lactate and pyruvate (Roth et al., 1988). Recent studies based on transcriptomic, proteomic and metabolomic analyses have revisited this simple model. The transcriptional analysis of parasites derived directly from infected patients identified three distinct clusters of malaria parasites. One cluster showed the induction of genes associated with glycolysis, amino acids and nitrogen metabolism, reflecting growth under anaerobic conditions typically observed in *in vitro* culture. In contrast, other samples presented the induction of genes associated with the TCA cycle, FA (fatty acid) metabolism and genes involved in glycerol uptake and metabolism [GK (glycerol kinase)] that reflected glucose starvation. This latter expression profile was reminiscent of the metabolic shift from glycolysis to mitochondrial metabolism in gametocytes (Daily et al., 2007). New samples from clinical patients as well as the results reported by Daily et al. (2007) were analysed by applying a statistical likelihood-based method designed to discriminate differential expression from cell-cycle-dependent expression (Lemieux et al., 2009). Such discrete differences were not observed in the new samples, suggesting that the previous observations were due to the variation of the sexual population in the samples (Lemieux et al., 2009). However, the absence of differences reported in the latter study could be masked by the cultivation of parasites in rich medium prior to the analysis (Wirth et al., 2009). Proteomic profiling hinted at an active TCA cycle that was more prominent in mosquito stages than in blood stages (Lasonder et al., 2008). Recently, more direct evidence based on metabolite profiling revealed the presence of TCA intermediates (malate, citrate, aconitate and α-ketoglutarate) consistent with the mitochondrion playing a significant role in the metabolism of trophozoites (Olszewski et al., 2009; Teng et al., 2009). It is plausible that the TCA cycle is functioning bidirectionally. This way, glutamine can be metabolized by the parasite to malate through two pathways: the reductive carboxylation pathway through citrate, yielding acetyl-CoA, and the oxidative pathway through succinate, yielding ATP and a precursor for haem biosynthesis (Olszewski et al., 2009).

Recently, two respiratory dehydrogenases were characterized in active mitochondria isolated from the rodent malaria parasite *P. yoelii yoelii*-infected RBCs (Kawahara et al., 2009). Taken together with the existence of a complete ATP synthase, these results suggest that the malaria parasite could indeed yield energy from oxidative phosphorylation (Uyemura et al., 2004; Mogi and Kita, 2009). In the RBC stages, two studies support the notion that the only essential function of the active mitochondrial electron-transport chain is to regenerate ubiquinone, which is required as the electron acceptor for dihydro-orotate dehydrogenase, an essential enzyme involved in *de novo* pyrimidine biosynthesis (Painter et al., 2007; Vaidya et al., 2008). However, these results are based on *in vitro* culture conditions and are restricted to the blood stages; substantial differences in the properties of asexual- and sexual-stage mitochondria can be anticipated. Recent results also described the mitochondrial localization of a functional ferrochelatase, an enzyme involved in the last step of haem biosynthesis in the mitochondrion, but its essential nature has not been demonstrated (Nagaraj et al., 2009). In *T. gondii*, oxidative phosphorylation was recently demonstrated to be essential for maintaining the ATP level in tachyzoites (Lin et al., 2009).

Irrespective of potential alternative energy sources, glucose remains the major energy source for the sexual stages of *P. falciparum* and is taken up by a single hexose transporter (PfHT; *P. falciparum* hexose transporter) localized at the parasite plasma membrane (Woodrow et al., 1999; Figure 1). PfHT mediates

the uptake of D-glucose and D-fructose and has been validated as a drug target (Joet et al., 2002; Joet et al., 2003). The essentiality of the *PfHT* gene has also been recently established (Slavic et al., 2010). *T. gondii* expresses a glucose transporter (TgGT1; *T. gondii* glucose transporter 1) at its plasma membrane (Pomel et al., 2008; Blume et al., 2009; Figure 2), which exhibits a higher affinity than PfHT for glucose ($K_m \approx 30$ μM versus 1 mM), probably reflecting an adaptation to its relatively glucose-poor environment (Joet et al., 2002). Three additional putative hexose/sugar permeases have been identified in the *T. gondii* genome, but the sugar specificity of these transporters still awaits further investigation (Blume et al., 2009).

Several invasive stages of the apicomplexans are able to glide on a substrate for a long period of time powered by the ATP-consuming motor myosin A and actin polymerization. Consequently, a considerable amount of ATP must be produced for their motility and to promote active host-cell entry. In a recent study, Pomel et al. (2008) investigated the significance of glucose for parasite gliding motility and proposed that glycolysis plays a major role as an energy provider. The glucose transporter TgGT1 is located at the parasite plasma membrane, and the glycolytic enzymes that are evenly distributed throughout the cytosol in intracellular parasites suddenly undergo a translocation to the pellicle during parasite egress from host cells (Figure 2). The authors interpreted this redistribution of glycolytic enzymes to the periphery of extracellular parasites as a means to locally enhance energy production to empower motility. The mechanism that triggers such a fast and selective spatial rearrangement remains unclear, but appears to be independent of intact F-actin and microtubules.

The interconnection between glycolysis and parasite motility is complicated by the fact that the glycolytic enzyme aldolase plays a dual function and also participates in invasion by bridging the actin cytoskeleton to micronemal proteins of the TRAP (thrombospondin-related anonymous protein) family. A recent study (Starnes et al., 2009) has described the dissection of the dual function of aldolase by creating a conditional knockout of this gene. The functional complementation of the mutant parasites with a series of point mutations in aldolase led to parasites that were either defective in glycolysis activity or in MIC2 (microneme protein 2) binding. The authors confirmed the vital dual role played by aldolase in energy production and host-cell invasion (Starnes et al., 2009).

The importance of glycolysis for invasion by, and intracellular replication of, *T. gondii* was also investigated by the functional analysis of the hexose transporters in this parasite (Blume et al., 2009). Deletion of the *TgGT1* gene established that TgGT1 serves as the main glucose transporter for tachyzoites. Rather unexpectedly, TgGT1 was shown not to be essential for parasite survival. The abrogation of glucose import from the host caused only a 30% decrease of intracellular growth and no alteration in gliding motility, invasion and virulence in mice. Interestingly, parasite mutants lacking TgGT1 are impaired in motility in minimal medium, but the defect can be restored if glutamine, but not glucose or pyruvate, is added to the medium. These results demonstrate that *T. gondii* tachyzoites can use glutamine as an alternative bioenergetic substrate to power motility (Figure 2B). Moreover, in the absence of TgGT1, oxidation of glutamine (glutaminolysis) meets the bioenergetic and biosynthetic demands for parasite intracellular growth and replication (Blume et al., 2009; Figure 2). This situation is analogous to cancer cells that fuel their metabolism preferentially with glucose; however, when glucose is no longer available, solid tumours use glutamine as an alternative energy source (Reitzer et al., 1979; DeBerardinis et al., 2007; Vander Heiden et al., 2009). Glutaminolysis produces α-ketoglutarate to maintain the TCA cycle and requires an active oxidative phosphorylation for ATP production (Figure 2). Such a versatile energy metabolism may explain how *T. gondii* replicates in almost all cell types regardless of their glucose level.

Carbon sources for lipid biosynthesis in *P. falciparum* and *T. gondii*

The high replication rate of apicomplexan parasites imposes a very high demand for lipids to expand the parasite endomembrane system and to adjust the size of the PV surrounding the continually growing number of parasites. *T. gondii* and *P. falciparum* are able to scavenge FAs from the host cell and serum (Charron and Sibley, 2002), but also have the capacity to produce FAs *de novo*. FA synthesis can be executed by the FASI (type I FA biosynthesis) pathway, consisting of a single huge polypeptide (>900 kDa), or by the proteobacterial FASII (type II FA biosynthesis) pathway,

involving a series of elongating enzymes acting in concert (Mazumdar and Striepen, 2007). Both pathways require acetyl-CoA as a precursor for FAS initiation and elongation. FASI is found in coccidians, including *T. gondii*, in *Cryptosporidium* spp., but is absent from the malaria parasites. In contrast, *T. gondii* and the *Plasmodium* spp. share FA elongases (Mazumdar and Striepen, 2007) and possess the complete set of enzymes involved in the FASII pathway. The FASII pathway is hosted by the apicoplast, the relic of a plastid originating from the secondary endosymbiosis of a red alga by the ancestor of the chromalveolates (Waller et al., 1998; Mazumdar et al., 2006). In the course of adaptation to parasitism, the apicomplexans have kept the plastid organelle for different purposes, but have not retained its photosynthetic capacity (Fleige et al., 2010).

The nature of the carbon source for FASII and its access to the organelle have recently been elucidated experimentally through the characterization of plastid triose transporters. Acetyl-CoA and malonyl-CoA are the essential precursors for FASII to be produced in the apicoplast. Following import, PEP (phosphoenolpyruvate) is metabolized into pyruvate and converted into acetyl-CoA by the action of a PDH (pyruvate dehydrogenase) complex found exclusively in this organelle (Foth et al., 2005; Figures 1 and 2A). A plastidic translocator is required to import PEP from the cytosol, thereby connecting glycolysis to lipid metabolism. In plants, sugars are transported in the plastids through membrane transporters that function as antiport systems exchanging phosphorylated C_3, C_5 and C_6 sugars. They can fuel the organelle by shuttling the glycolytic products of glucose acquired from the host into the plastid. A single APT (apicoplast phosphate translocator), TgAPT1, was identified in the *T. gondii* genome and localized to multiple membranes of the organelle (Fleige et al., 2007; Karnataki et al., 2007; Figure 2A). Biochemical results demonstrated that TgAPT1 is able to transport various substrates, including triose phosphate and PEP required for the apicoplast FASII and DOXP (1-deoxy-D-xylulose 5-phosphate) isoprenoid-synthesis pathways (Brooks et al., 2010). The conditional disruption of the *TgATP1* gene demonstrated that the transport of metabolites to the plastid is essential for parasite survival and hence confirmed the previously reported essential nature of the FASII pathway in *T. gondii* (Mazumdar et al., 2006). In addition, the stronger phenotpye observed with APT1 depletion compared with ACP (acyl-carrier protein) depletion suggests a second critical function for this transporter in substrate import for the DOXP isoprenoid-synthesis pathway (Brooks et al., 2010). In contrast, *P. falciparum* possesses two distinct genes coding for potential transporters that localize differentially to the inner [PfiTPT (*P. falciparum* inner membrane triose phosphate transporter) or PfAPT1] and the outer membrane [PfoTPT (*P. falciparum* outer membrane triose phosphate transporter) or PfAPT2] of the apicoplast respectively (Mullin et al., 2006; Figure 1). The substrate preferences of these transporters were assessed recently using a novel cell-free assay system and suggest that they act in tandem to transport phosphorylated metabolites [PEP, DHAP (dihydroxyacetone phosphate) and 3PGA (3-phosphoglycerate)] from the parasite cytosol into the plastid (Lim et al., 2010). The imported PEP is then transformed into pyruvate in the apicoplast, thanks to the action of the PKII (pyruvate kinase type II) characterized both in *T. gondii* and *P. falciparum* (Saito et al., 2008; Maeda et al., 2009).

The importance of FASII for the survival of *T. gondii* tachyzoites has been well established, despite the presence of cytosolic FASI and FA elongases that obviously fail to compensate for the depletion of TgACP both *in vitro* and *in vivo* (Mazumdar et al., 2006). On the contrary, three recent studies demonstrated that *de novo* FA synthesis is dispensable in the RBC stages of the malaria parasites (Yu et al., 2008; Tarun et al., 2009; Vaughan et al., 2009). Unexpectedly, the absence of FASII does not interfere with gametogenesis, and the mutants progress normally through the mosquito stages and successfully establish infection in hepatocytes. However, the late-liver-stage development is severely blocked and fails to form mature exo-RBC merozoites that normally initiate blood-stage invasion. In consequence, the intra-RBC stages can compensate for the absence of *de novo* FA biosynthesis either by scavenging host FAs or by relying on elongases for FA synthesis, whereas the reason why the liver-stage merozoites cannot has not been elucidated. The potential need for unusual FA species in the liver stage has been recently postulated to explain this dependency (Tarun et al., 2009). The *de novo* FAs produced in the apicoplast might indeed be exported and used elsewhere in the parasite (Ralph et al., 2004).

Further experimental evidence suggests that both *Plasmodium* spp. and *T. gondii* are versatile in obtaining carbon from sources other than glucose. *P. falciparum* encodes a single AQP (aquaglyceroporin), PfAQP, permeable to glycerol and water. PfAQP is expressed at the parasite periphery in all blood stages (Figure 1). Host glycerol uptake seems to take place through the major human AQP, hAQP9 (Liu et al., 2007), and then PfAQP provides direct access to blood serum glycerol via the erythrocyte cytosol, allowing ATP generation and phospholipid synthesis after phosphorylation via GK (Hansen et al., 2002). A *P. berghei* mutant lacking AQP is still viable, but is less virulent in mice, indicating that glycerol uptake may be important for normal proliferation of the parasite (Promeneur et al., 2007). Interestingly, a recent study has demonstrated that, in *P. falciparum*, in addition to lactate, glycerol is an unexpected end-product of glucose catabolism (Lian et al., 2009). Glycerol uptake through the host AQP and then throught the parasitic AQP should become relevant when glucose is limited, for example during the vector stages (Beitz, 2007). PfGK phosphorylates glycerol, which can then be converted into dihydroxyacetone-3-phosphate by glyceraldehyde-3-phosphate dehydrogenase and fed into glycolysis (Figure 1). Recently, the deletion of PfGK demonstrated that exogenous glycerol is not an important carbon source for the *P. falciparum* blood-stage parasite *in vitro* (Schnick et al., 2009).

Rapid dividing *T. gondii* tachyzoites are likely to depend on cytosolic acetyl-CoA production to fuel the FASI pathway. The parasite possesses a cytosolic ACL (ATP citrate lyase) to produce acetyl-CoA and OAA (oxaloacetate) (Seeber et al., 2008). OAA can then be used during gluconeogenesis to generate glucose 6-phosphate in the cytosol. In cancer cells, citrate is preferentially exported to the cytosol via a tricarboxylate transporter, where it is cleaved by ACL to produce cytosolic acetyl-CoA and regenerate OAA, that is then reduced to malate, which can return to the mitochondria to complete the cycle. ACL knockdown limits cancer-cell proliferation, showing that ACL is therefore a key enzyme integrating glucose and lipid metabolism (Hatzivassiliou et al., 2005). In apicomplexans, the absence of a mitochondrial PDH complex poses a dilemma for the generation of acetyl-CoA and accomplishment of a functional TCA cycle. Production of acetyl-CoA via β-oxidation is not supported by any direct biochemical evidence. Database mining of the *P. falciparum* genome failed to identify enzymes involved in this pathway (Carlton et al., 2002), In contrast, the *T. gondii* genome revealed the presence of genes involved in lipid oxidation (acyl-CoA dehydrogenase, enoyl-CoA hydratase, hydroxyacyl-CoA dehydrogenase and ketoacyl-CoA thiolase), indicating that this pathway might take place (Seeber and Soldati, 2007). However, β-oxidation starts once fatty acids have been imported into the mitochondrial matrix by carnitine acyltransferases that seem to be missing in the *T. gondii* genome.

Alternatively, *T. gondii* possesses all the enzymes involved in the degradation pathway of BCAAs (branched-chain amino acids), which leads to the production of acetyl-CoA in the mitochondrion (Seeber et al., 2008). Incidentally, the BCAA degradation pathway simultaneously produces propionyl-CoA, a toxic metabolite able to inhibit cell growth. Bacteria and fungi detoxify propionyl-CoA via the MCC (methylcitrate cycle) pathway that leads to the α-oxidation of propionate into pyruvate (Brock et al., 2002; Brock, 2005). The complete set of enzymes involved in the MCC is present in the coccidians, including *T. gondii*. Consistent with the absence of FASI in *P. falciparum*, the BCAA and MCC pathways are not found in the malaria-parasite genome, although the BCKDH (branched-chain ketoacid dehydrogenase) is preserved (Seeber et al., 2008). This complex is localized in the mitochondria and has been shown to be lipoylated (Foth et al., 2005; Gunther et al., 2005; McMillan et al., 2005; Gunther et al., 2007). The physiological role of this complex is uncertain, but it might contribute to the generation of acetyl-CoA in the mitochondrion (Seeber et al., 2008). The affinity of BCKDH for pyruvate still needs to be assessed experimentally and, so far, the complex appears to be dispensable for the *P. berghei* erythrocytic stages (V. Polonais and D. Soldati-Favre, unpublished work).

Intriguingly, *T. gondii* tachyzoites express at the plasma membrane a transporter that shares resemblance to a mitochondrial citrate transporter allowing the import of citrate and/or succinate, precursors for acetyl-CoA for FASI (T. Fleige and D. Soldati-Favre, unpublished work). This transporter is closely related to INDY (I'm not dead yet) in *Drosophila melanogaster* (Rogina et al., 2000; Inoue et al., 2002). This transporter is found in mammals (Pajor, 1999) and in *Caenorhabditis elegans* (Fei et al., 2003) and mediates

high-affinity flux of sodium dicarboxylates and TCA intermediates. The importance of TgINDY for parasite survival, its biochemical properties and substrate specificities is awaiting further investigation.

Conclusions

During the past decade, genome, transcriptome and proteome analyses applied to apicomplexans has helped immensely with putting forward novel hypotheses concerning the energy and carbon metabolism governing growth of these parasites (Chaudhary and Roos, 2005). The accessibility of *Plasmodium* spp. and *T. gondii* to genetic manipulation is offering a powerful opportunity to challenge, and ultimately refute or confirm, some of these postulates. It is now timely for parasitology to enter into the area of metabolomics and to combine the functional analyses with a global and quantitative measurement of all metabolites. Ultimately, the systematic reconstruction of genome-scale models of apicomplexan central metabolism should be instrumental in assessing essential pathways and identify novel targets for intervention.

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