Crosstalk between purine nucleotide metabolism and mitochondrial pathways in *Plasmodium falciparum*

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Metabolism is known for its intricate adjustments to meet the needs of an organism. Due to demands of adaptation, parasite metabolic pathways are greatly altered from those of their hosts. A key difference in metabolic pathways in *Plasmodium* spp, during the intra-erythrocytic stages, pertains to energy metabolism with the absence of a major role for the mitochondria in adenosine triphosphate (ATP) generation. In most organisms there exists a tight link between nucleotide and energy metabolism. An important feature of purine nucleotide metabolism in *Plasmodium falciparum* is the absence of the *de novo* pathway, with purine requirements being completely met by the salvage pathway. Presence of the enzymes adenylosuccinate lyase, adenylosuccinate synthetase and adenosine monophosphate (AMP) deaminase, involved in AMP metabolism, suggests the existence of a functional purine nucleotide cycle (PNC) in *P. falciparum* with fumarate, a tricarboxylic acid (TCA) cycle intermediate, and ammonia being the net output from the cycle. In the absence of a conventional TCA cycle, the fate of fumarate generated from PNC merits examination. In this review we cover ATP generation through glycolysis, key features of the TCA cycle, the role of electron transport chain and the link between PNC and the mitochondrion. Recent studies using genetic approaches highlight unexpected features that were hitherto unknown with respect to these pathways and these are also summarized in this review.

Keywords: Fumarate, glycolysis, mitochondrion, *Plasmodium falciparum*, purine nucleotide cycle, TCA cycle.

Introduction

PLASMODIUM FALCIPARUM, the causative agent of cerebral malaria completes its life cycle in two hosts: mosquito and human. Growth and reproduction in two varied habitats necessitates the adaptation of the organism to completely different environments. This has led to the evolution of metabolic pathways in the parasite that are optimized to utilize available nutrients in the two hosts[1].

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The completion of the genome sequence has contributed, to a great extent, to our understanding of the metabolism in *P. falciparum*[2], wherein several key differences are evident when compared to free-living organisms. Noticeably, *P. falciparum* lacks two key pathways, namely amino acid and purine nucleotide biosynthesis. Despite the presence of genes for the tricarboxylic acid (TCA) cycle enzymes and the mitochondrial electron transport chain (ETC) complexes, biochemical studies attribute glycolysis to being the key pathway for adenosine triphosphate (ATP) generation in intraerythrocytic (IE) parasites[3]. With the source of acetyl coenzyme A (acetyl-CoA), the metabolite that initiates the TCA cycle, apparently missing in the mitochondrion of *Plasmodium* spp, α-ketoglutarate, generated from glutamine, was observed to feed in and initiate a branched TCA pathway in the IE stages of the parasite[4]. Adenosine monophosphate (AMP) and guanosine monophosphate (GMP) synthesis in the IE stages is via the salvage of hypoxanthine and adenosine from the human host. AMP is synthesized from inosine monophosphate (IMP) by the sequential action of adenylosuccinate synthetase (ADSS) and adenylosuccinate lyase (ASL). AMP deaminase (AMPD) deaminates AMP back to IMP. The reactions catalysed by these three enzymes constitute the purine nucleotide cycle (PNC). Figure 1 shows a schematic representation of PNC that comprises the enzymes ADSS, ASL and AMPD. In exercising skeletal muscles, the fumarate generated from PNC feeds into the TCA cycle and contributes to increase in ATP levels[5]. However, the role of PNC in *P. falciparum* is not fully understood. In this review we provide an overview of the literature on mitochondrial pathways, purine nucleotide metabolism and hypothesize on the link between the two in *P. falciparum*.

Glycolysis in *P. falciparum*

It was observed that *P. falciparum*-infected erythrocytes take up 100 times more glucose than the uninfected erythrocytes[6]. The increased consumption is attributed to both the transporters and the enzymes of the parasite compartment. The glucose uptake is through a high-affinity

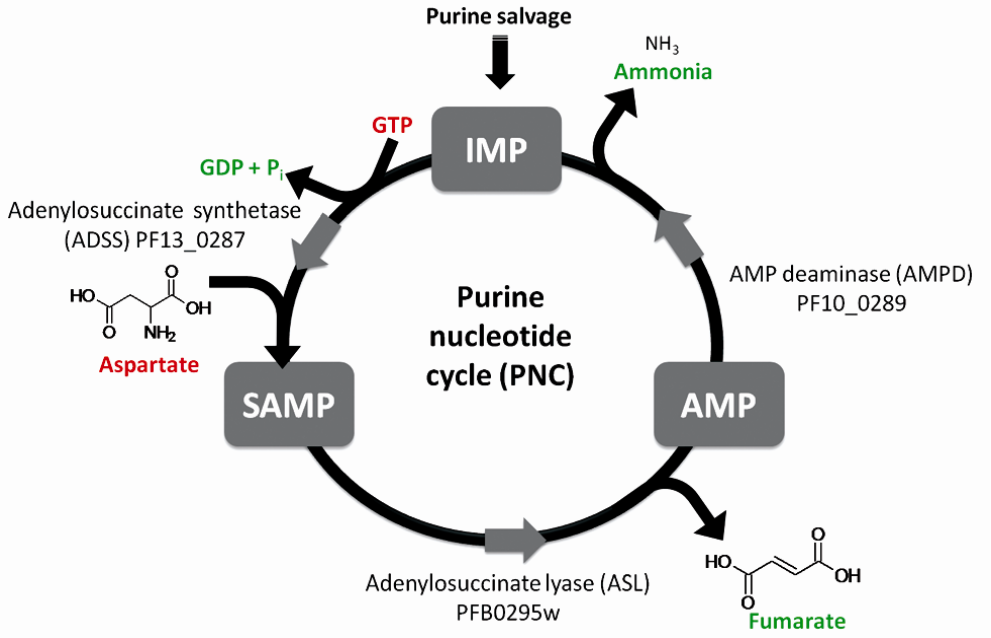

Figure 1. Purine nucleotide cycle (PNC). The enzymes and three reactions involved in the cycle are shown. The accession numbers correspond to the enzymes from *Plasmodium falciparum*. One molecule each of fumarate, ammonia and inorganic phosphate are the net products formed from the complete operation of the cycle. The substrate consumed in the process is shown in red and net products formed are shown in green. SAMP, Succinyl-AMP; AMP, adenosine monophosphate; IMP, inosine monophosphate.

facilitative hexose transporter (PfHT)[7]. The transporter is expressed throughout the lifecycle of the parasite and through the use of inhibitors it has been shown to be essential for the intraerythrocytic parasite development[8]. Unlike the human glucose transporter, glut1, which is highly specific for glucose, PfHT can take in fructose as well[7]. This differential substrate specificity has enabled the design of drugs which specifically target the parasite transporter[9]. Furthermore, the key regulatory enzymes of glycolysis, hexokinase and phosphofructokinase are reported to have low K_m values for their substrates[10]. A thorough study on the mechanism of enhanced glycolytic flux is still missing. Metabolic labelling studies show that bulk of the glucose is metabolized to lactate[11–13]. The enzyme lactate dehydrogenase (LDH) is essential for the parasite and is highly expressed[14]. The crystal structure of this enzyme shows differences from that of the host suggesting functional distinctions[14,15]. One of the key functional differences is that unlike LDH from other organisms, the plasmodial enzyme is not inhibited by high concentrations of pyruvate, an adaptation reflective of the microaerophilic nature of the organism[16]. The conversion of pyruvate to lactate enables the oxidation of reduced nicotinamide adenine dinucleotide (NADH), thereby providing sufficient nicotinamide adenine dinucleotide (NAD^+) for the continuous operation of glycolysis. Interestingly, glycerol was found to be one of the metabolites formed from glucose in the IE parasite[11]. This implies the possible presence of a functional glycerol-3-phosphate shuttle in *P. falciparum* and that the parasite has alternate mechanisms to reoxidize cytosolic NADH. However, the enzymes involved in the formation of glycerol are not known. Lian *et al.*[11] have suggested the role of glycerol 3-phosphatase (unannotated) or glycerol kinase, operating in the reverse direction (thermodynamically unfavourable) as in trypanosomes[17,18], as probable enzymes involved in the formation of glycerol. An alternative explanation suggests that a non-specific acid phosphatase can act upon glycerol 3-phosphate and form glycerol[19]. Further experimentation would probably highlight the metabolic significance of glycerol production in the parasite.

Source of acetyl CoA and TCA cycle

The absence of incorporation of label from glucose into TCA cycle intermediates[11,12,20] implies that glycolysis is disconnected from the TCA cycle in the parasite. This is further supported by the finding that the pyruvate dehydrogenase (PDH) complex in *P. falciparum* localizes to the apicoplast[21,22] and not to the mitochondrion. Pei *et al.*[23] showed that deletion of the E1α or E3 subunit genes of the PDH complex in the rodent malaria parasite, *Plasmodium yoelii* caused no defect in blood, mosquito or early liver stage development. However, the knockout parasites could not initiate the blood-stage infection as they were unable to develop into exo-erythrocytic mero-

zoites. This phenotype is similar to that observed for deletion of genes involved in type II fatty acid synthesis (FAS II)[24,25]. Thus, these results indicate that the sole role of PDH is probably to provide acetyl-CoA for FAS II rather than the TCA cycle.

β-Oxidation pathway of fatty acids in the mitochondria is another potential source of acetyl-CoA, but the genes for this metabolic process have not been annotated in any of the Plasmodial genomes sequenced thus far[2,26]. Other sources of acetyl-CoA are branched-chain ketoacid dehydrogenase complex and carnitine acetyl transferase. The annotated genome of *P. falciparum* does not have all the components necessary for the complete conversion of ketoacids to acetyl-CoA[27]. Though an annotation for carnitine acetyl transferase is missing, carnitine has been shown to provide growth support for erythrocyte-free parasites in culture[28]. Whether the growth advantage is due to the activity of carnitine acetyl transferase is yet to be tested. Thus, the parasite mitochondrion seems to be devoid of acetyl-CoA to fuel the TCA cycle.

Examination of the fate of added ^{13}C-glutamine by liquid chromatography–mass spectrometry (LC–MS) analysis showed that the TCA cycle in *P. falciparum* is branched with α-ketoglutarate feeding in and initiating the pathway[4]. Although this seminal discovery has put an end to the long standing controversy about the existence of this pathway, it has fuelled more queries about its nature and essentiality. The added glutamine must be converted to α-ketoglutarate through glutamate. The conversion of glutamine to glutamate can be brought about by various amidotransferases (e.g. carbamoyl phosphate synthetase, GMP synthetase, glutamine : oxoglutarate amidotransferase, etc.) that are present and expressed during the IE stages of the parasite[2,29–31]. Glutamate dehydrogenase (GDH) or aminotransferases were speculated to facilitate the subsequent conversion of glutamate to α-ketoglutarate. However, in a recent report by Storm *et al.*[32], using *gdha* knockout strain of *Plasmodium berghei*, the non-involvement of this gene in the generation of α-ketoglutarate was shown. Also, through the use of the inhibitor L-cycloserine, it was shown that alanine and asparatate aminotransferases are also not involved in facilitating this conversion[32]. In this context a study on the role of GDHb and GDHc in generating α-ketoglutarate is warranted.

The NADP-dependent isocitrate dehydrogenase (IDH) catalyses the first reaction in the reductive branch of the non-canonical TCA pathway[4]. The reaction is reversible and the ratio of $NADP^+$: NADPH in the mitochondrion determines the direction of the reaction, with lower ratios favouring the reductive reaction. Glutamate dehydrogenase (GDH) was thought to be a major source of NADPH in the parasite. With the generation of a viable $GDHa^-$ strain[32], an alternate source of NADPH must be present to drive the reductive reaction of IDH. The alternate source of NADPH could be pyridine transhydrogenase or GDHc. An earlier study by Wrenger and Muller[33] has provided kinetic parameters for IDH catalysing the oxidative reaction. However, the kinetic features of the reductive reaction catalysed by this enzyme have not been examined. Other aspects of the branched TCA pathway such as: (1) the enzyme for the conversion of citrate to oxaloacetate (OAA) and acetyl-CoA (attributed to ATP-dependent citrate lyase activity in the erythrocyte cytosol) and (2) transporters for acetyl-CoA in mitochondria are still unclear. Though some functional aspects such as source of succinyl-CoA, guanosine triphosphate (GTP) and acetyl-CoA have been attributed to this branched pathway, their essentiality in different stages of the parasite life cycle has to be validated by gene knockout studies.

The role of mitochondrion in *P. falciparum*

Plasmodium species during the intraerythrocytic phase of their life cycle are known to possess a single mitochondrion. The mitochondrion among plasmodial spp. is heterogenous with respect to size, shape, behaviour upon subcellular fractionation, extent of internal structures, etc.[34–37]. Obvious differences are the extent of oxidative phosphorylation and the alternative oxidase activity in different species. Rodent parasites, *P. yoelii* and *P. berghei* seem to have a higher rate of oxidative phosphorylation compared to *P. falciparum*[35–37]. Unlike other species in the genus, *P. falciparum* is reported to have an alternative oxidase activity[38].

Though mitochondria underwent a reductive evolution in apicomplexan spp, their role seems to be essential for the survival of the parasites. The essentiality of this organelle is due to the many functions it performs, viz. (1) it is the source of succinyl-CoA essential for heme biosynthesis; (2) it is the source of orotate, an intermediate in *de novo* pyrimidine synthesis[39] and (3) iron sulphur cluster biosynthesis[27], which is essential for the activity of many enzymes. Though validated as a drug target for malaria, a clear understanding of the various functional aspects of the mitochondrion in *P. falciparum* is still lacking. Earlier studies have demonstrated that glycolysis and ETC are uncoupled and there is little, if any, ATP contribution by oxidative phosphorylation[40]. Nevertheless, mitochondrial membrane potential has been proven to be an essential component for the survival of the parasite in the intraerythrocytic stages. The role of the membrane potential apart from being vital for respiration is also implicated in the transport of small molecules and proteins across the inner mitochondrial membrane[27].

Electron transport chain

P. falciparum has all the five respiratory complexes, though they are different from those of the mammalian

mitochondria. Complex I is rotenone insensitive and non-proton pumping and belongs to class II NADH dehydrogenase[41]. Complex II comprising succinate dehydrogenase converts succinate to fumarate. The earlier proposed quinol fumarate reductase activity (which converts fumarate to succinate) in the IE stages has been disproved by various metabolic labelling studies in the recent literature[4,42]. Moreover, the enzyme isolated from *P. yoelii* behaves similar to the human enzyme and gets inhibited by atpenin, a mammalian complex II inhibitor[34]. Complex III, the cytochrome bc_1 complex (proton pumping), is the only complex which generates oxidized ubiquinone that is essential for the activity of many mitochondrial dehydrogenases. Various classes of inhibitors like naphthoquinones, pyridines, acridones, aridinediones and quinolones target the Q_0 site of complex III, resulting in collapse of the membrane potential[43–45]. Complex IV which consists of cytochrome *c* oxidase (proton pumping) is sensitive to cyanide inhibition[46–48]. Complex V in the parasite is still not well characterized, though recent bioinformatic analysis provides annotation for all subunits of the F_1 and F_0 complexes[49]. For details about these complexes, the reader may refer elsewhere[27,50–53].

Total mitochondrial membrane potential, Δp, is the sum of the potential contributed by ΔpH (difference in concentration of H^+ ions) and $\Delta p\Psi$ (electron transfer potential). Complexes III and IV, that are proton pumping contribute to ΔpH, whereas complexes I to IV contribute to $\Delta p\Psi$. The contribution from alternative oxidase in *P. falciparum* is still not validated though 25% of the O_2 consumption is attributed to alternative oxidase activity[38]. Other components like the ATP/adenosine diphosphate (ADP) translocator are also hypothesized to contribute through ΔpH to the membrane potential[53]. The relative contribution of these components to total mitochondrial membrane potential is still not determined in any *Plasmodium* spp.

Mitochondrial dehydrogenases and their essentiality

There are at least four dehydrogenases, viz. NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), dihydroorotate dehydrogenase (DHODH) and glycerol 3-phosphate dehydrogenase associated with the mitochondrion of *Plasmodium*[35,41,49,54]. In addition, the parasite also has a malate dependent quinone oxidoreductase (MQO)[37]. DHODH, which catalyses the conversion of dihydroorotate to orotate, is an essential enzyme involved in pyrimidine biosynthesis. It belongs to type 2 class of DHODH and is dependent on the availability of oxidized ubiquinone for its function and hence, is dependent on complex III for its activity. Supplementing the parasite with yeast DHODH (yDHODH), a type 1 fumarate-dependent DHODH, makes the parasite resistant to atovaquone, a ubiquinone analogue that inhibits complex III by binding to cytochrome *b* subunit. However, different strains of transgenic *P. falciparum* expressing yDHODH from a single copy of the gene integrated into their genome show variation in susceptibility to atovaquone[55]. This unexpected finding suggests that there is a strain-dependent variation in the essentiality of dehydrogenases other than DHODH. In this context, there is a need for gene knockout studies to conclusively prove the role of these dehydrogenases in different strains of the parasite. The non-proton pumping complex I in *P. falciparum* referred to as NADH dehydrogenase 2 (NDH2), has been claimed to be essential based on *in vitro* inhibition of the parasite by various inhibitors[41,56]. In fact, extensive efforts have been put towards development of inhibitors directed to the inhibition of *P. falciparum* NDH2 activity. However, a recent study excluded the essentiality of NDH2 in *P. berghei*[57]. Knockout of NDH2 in *P. berghei* showed that the strain was viable with an intact mitochondrial membrane potential and normal virulence. However, NDH2-deficient strains were not able to undergo sporogony in the mosquito stages and showed arrest at the ookinete stage though mitochondrial membrane potential in this stage was not lost. The authors suggest that processes involving high ATP requirement in the parasite during oocyst development, such as organelle disposal, are severely affected.

Alternate oxidase

Cyanide-independent respiration in *P. falciparum* suggests the existence of alternate oxidase[38]. That atovaquone does not completely collapse the mitochondrial membrane potential indicated that a portion of the membrane potential is insensitive to atovaquone treatment[39]. Proguanil, a dihydrofolate reductase (DHFR) inhibitor was however able to collapse this potential by an unknown mechanism[58]. It has been reported that alternate oxidase inhibitors, salicylhydroxamic acid (SHAM) and propyl gallate, potentiate the activity of atovaquone[59]. A recent study has examined the proteomic changes in *P. falciparum* caused by treatment with SHAM[60]. Upon drug treatment, among other changes observed, a key change was seen in upregulation of the levels of some glycolytic enzymes. Alternate oxidase from plants is well characterized and the enzyme is active as a homodimer. It is known that organic acids (like pyruvate) allosterically activate alternative oxidase by promoting its dimerization[61]. Increased pyruvate formation upon activation of glycolysis, is suggested to counteract the inhibitory effect of SHAM in *P. falciparum*. It was also observed that under hyperoxic conditions there was a dramatic drop in the inhibitory concentration (IC_{50}) of SHAM, suggesting that the metabolic adaptation to oxygen-rich environment is sensitive to SHAM. However, sequences with homology

to altenate oxidases from other organisms have not been identified in *P. falciparum*.

Apicoplast and mitochondrial connection

The apicoplast and the mitochondrion in *P. falciparum* are always seen to lie in close contact with each other. Hence, transport of metabolites across these organelles was hypothesized[62]. The key pathways in apicoplast are type II fatty acid biosynthesis, iron sulphur cluster biosynthesis, isoprenoid biosynthesis and a portion of heme biosynthesis. Using a chemical genetics approach, Yeh and Derisi[63] have shown that isoprenoid biosynthesis is probably the only key function of the apicoplast. Inhibition with the antibacterial agent, fosmidomycin that targets isoprenoid biosynthesis was shown to be overcome by supplementing the parasite cells with isopentenyl pyrophosphate (IPP). The IPP-rescued parasites seemed to be devoid of the apicoplast, while the mitochondrion was intact as evident by the measurement of mitochondrial/nuclear DNA ratio and Mito Tracker staining. In this context, it will be interesting to examine the metabolic activities in the mitochondrion in the absence of apicoplast, as the two organelles are closely associated in the parasite. The susceptibility of transgenic *P. falciparum* strain expressing yDHODH (fumarate-dependent and ubiquinone-independent) to fosmidomycin suggests that IPP apart from serving as precursor for menaquinone/ubiquinone biosynthesis, might serve other essential roles. However, it is not possible to rule out the necessity of ubiquinone requirement for other dehydrogenases. This study also shows that apicoplast-generated acetyl-CoA is not essential for the parasite survival, though it might be compensated for by the mitochondrial acetyl-CoA. This needs further investigation.

Purine nucleotide metabolism in *P. falciparum*

The purine and pyrimidine requirements of the parasite are met by dedicated pathways in the organism. The purines are salvaged from the host[2,64], whereas the pyrimidines are synthesized *de novo* in the parasite[20]. Flux balance analysis of *P. falciparum* metabolic network showed that the purine salvage pathway and its enzymes are essential for parasite survival[65]. Most of the enzymes in the purine salvage pathway have been biochemically and kinetically characterized[66–68]. The main source of purine in the intraerythrocytic stages of *P. falciparum* is adenosine and hypoxanthine. Once taken into the parasite cytosol, adenosine is converted to inosine and thereafter to hypoxanthine by sequential action of the essential enzymes, adenosine deaminase and purine nucleoside phosphorylase[69–72]. The hypoxanthine generated is converted to IMP by the action of hypoxanthine guanine phosphoribosyltransferase (HGPRT). IMP is the nodal point from where both AMP and GMP are synthesized. AMP is generated by the sequential action of ADSS and ASL. The AMP generated is also converted back to IMP by the action of AMPD, as shown by the incorporation of ^{33}P into IMP when parasites are fed with [^{33}P]–AMP[73]. This pathway of conversion of IMP to succinyl-AMP (S-AMP) and further to AMP that is converted back to IMP constitutes the PNC. All the enzymes involved in the PNC are expressed during the intraerythrocytic stages of the parasite[30]. The stoichiometry of the cycle is such that for every molecule of aspartate and GTP consumed, the net products formed are: (1) one molecule of fumarate, (2) one molecule of GDP and inorganic phosphate, and (3) one molecule of ammonia. The presence of PNC in *P. falciparum* suggests the need for regulation of AMP levels, and a possible role for fumarate and ammonia in parasite metabolism.

Examination of the annotated genome databases of various apicomplexans permitted the comparison of AMP metabolism and the possible existence of PNC in these organisms. A list of genes related to PNC in various apicomplexans is provided in Table 1. Interestingly, the complete PNC is present in *Toxoplasma gondii*, *Neospora caninum*, *Eimeria tenella* and all species of *Plasmodium*. Though ADSS and ASL are absent in *Cryptosporidium*, AMPD is present and AMP formation is catalysed by adenosine kinase from the precursor adenosine[74]. The presence of AMPD in this organism suggests that regulation of AMP levels is critical and the ammonia generated may have metabolic significance. *Theileria* and *Babesia*, on the other hand, possess ADSS and ASL, but lack AMPD. In these organisms the fate of fumarate generated is an aspect that warrants investigation.

Table 1. Summary of the enzymes of purine nucleotide cycle present in apicomplexans[a]

Organism	ADSS	ASL	AMPDA
Cryptosporidium parvum	–[b]	–	√[c]
Cryptosporidium hominis	–	–	√
Cryptosporidium muris	–	–	√
Plasmodium falciparum	√	√	√
Plasmodium vivax	√	√	√
Plasmodium yoelii	√	√	√
Plasmodium berghei	√	√	√
Plasmodium chabaudi	√	√	√
Plasmodium knowlesi	√	√	√
Toxoplasma gondii	√	√	√
Neospora caninum	√	√	√
Eimeria tenella	√	√	√
Theileria annulata	√	√	–
Theileria parva	√	√	–
Babesia bovis	√	√	–

[a]Sequence search was done using BLAST in the in NCBI protein database. The retrieved hits were confirmed in EupathDB and GeneDB database. The sequence of *P. falciparum* 3D7 (AMPD, ADSS, ASL) were used as BLAST query; [b]Absence of protein homologue; [c]√Presence of protein homologue. AMPD, AMP deaminase; ADSS, adenylosuccinate synthetase; ASL, adenylosuccinate lyase.

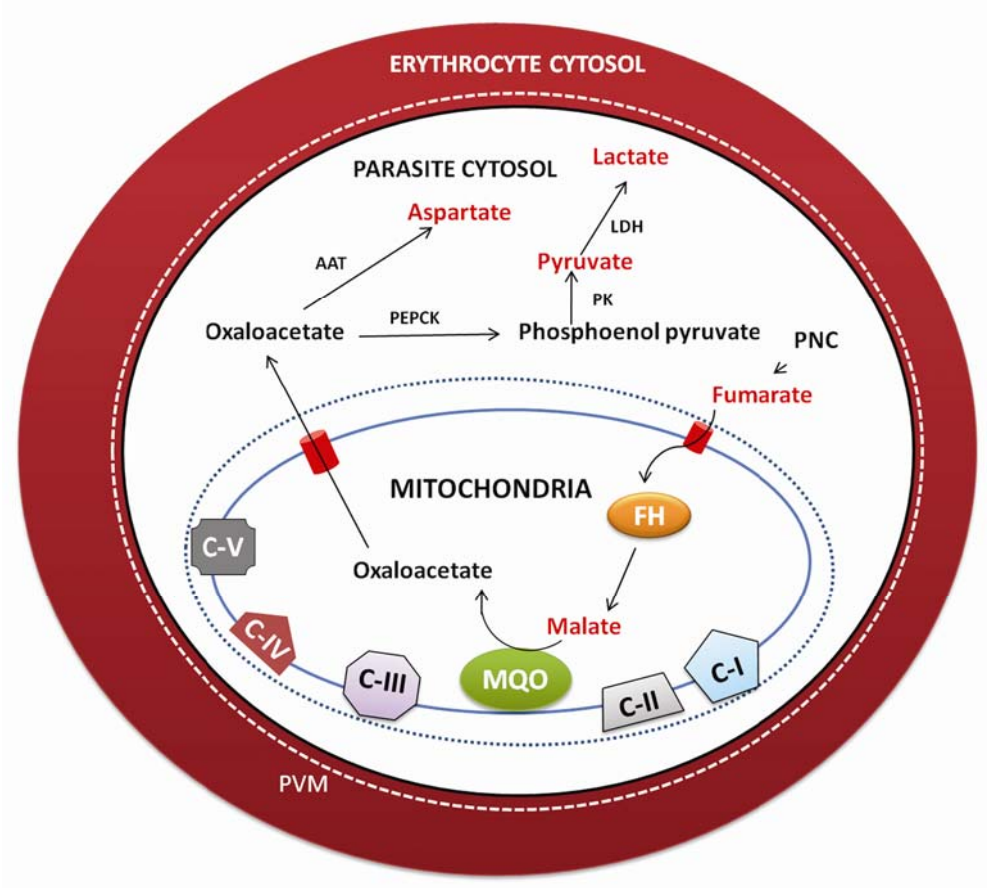

Figure 2. Scheme showing the fate of fumarate in *P. falciparum*. Fumarate produced in the cytosol by PNC is shuttled into the mitochondrion where it is converted into oxaloacetate (OAA) through the involvement of fumarate hydratase (FH) and malate quinone oxidoreductase (MQO). The OAA transported into the cytosol serves as the precursor for aspartate and pyruvate. C I-V, Complex I–V; MQO, malate quinone oxidoreductase; AAT, aspartate aminotransferase; LDH, lactate dehydrogenase; PEPCK, phosphoenol pyruvate carboxykinase; PK, pyruvate kinase; PNC, purine nucleotide cycle; FH, fumarate hydratase.

Fate of fumarate generated by PNC in P. falciparum

Protozoan parasites are known to secrete organic acids such as lactate, malate, succinate, fumarate, etc.[75]. Through the use of ^{14}C-labelled aspartate, recent studies have shown that fumarate generated through PNC is not secreted as a metabolic waste in the IE stages of *P. falciparum*[42]. Using intraerythrocytic *in vitro* cultures of *P. falciparum*, ^{13}C-labelled fumarate (2,3-^{13}C fumarate) supplemented in the medium was shown to be metabolized to pyruvate and aspartic acid[42]. The pathway for fumarate metabolism is shown in Figure 2. As shown in the figure, the key intermediate for both aspartic acid and pyruvate formation, from fumarate, is OAA. The fumarate hydratase activity which brings about conversion of fumarate to malate has been shown to be localized to the mitochondrion in the parasite. The subsequent conversion of malate to OAA by MQO was validated through the use of atovaquone. Atovaquone-treated parasites failed to accumulate ^{13}C-labelled aspartate, suggesting the role of mitochondrial MQO and not cytosolic malate dehydrogenase in the generation of OAA from malate. The conversion of OAA to aspartate is brought about by aspartate aminotransferase (AAT). Recombinant *P. falciparum* AAT has been shown to catalyse the reaction efficiently in both directions and localize to the cytosol[42,76]. The conversion of OAA to pyruvate must be through phosphoenolpyruvate carboxykinase (PEPCK) followed by pyruvate kinase. This conversion of fumarate to aspartic acid and pyruvate is compartmentalized between the mitochondrion and the cytosol. The whole process therefore, requires the operation of shuttles that transport fumarate into the mitochondria and OAA out from this organelle.

Significance of PNC in P. falciparum

Schematically shown in Figure 3 are the possible roles for fumarate and ammonia generated by PNC and their crosstalk with other pathways in *P. falciparum*. Major role of PNC has been attributed to meeting the increased ATP demand and/or maintaining acid–base homeostasis

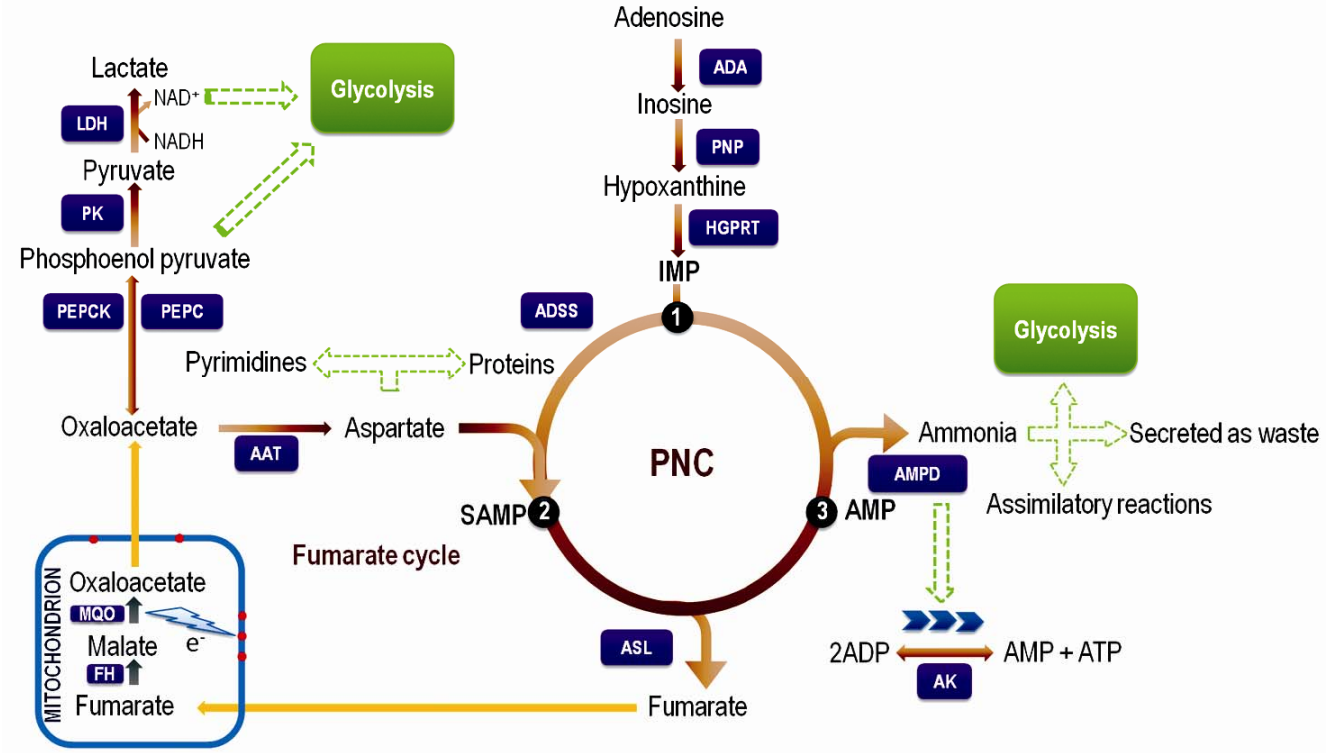

Figure 3. Crosstalk of PNC with other metabolic pathways in *P. falciparum*. Numbers 1–3 in PNC refer to the metabolites inosine monophosphate (IMP), SAMP and adenosine monophosphate (AMP) respectively. Brown arrows, enzymatic reactions in cytosol; grey arrows, enzymatic reactions in mitochondrion; orange arrows, transport processes; broken green arrows, possible role of metabolites (enzyme substrates or regulators); violet boxes, enzymes catalysing the reaction; lightning bolt, electron donation to ubiquinone; red filled circles, ubiquinone molecules and chevron arrows, directionality of the reaction. PEPC, Phosphoenolpyruvate carboxylase; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; HGPRT, hypoxanthine guanine phosphoribosyltransferase; AK, adenylate kinase. Expansions for the remaining abbreviations are provided in the legend to Figures 1 and 2.

in the cell. This is achieved by three ways, viz. by shifting the equilibrium of adenylate kinase, by altering the flux of metabolites through TCA cycle reactions and by the release of ammonia. Adenylate kinase directly regulates the ATP pool and the directionality of the reaction catalysed by this enzyme is sensitive to levels of AMP[77,78]. In PNC, AMPD acts on AMP and converts it back to IMP, thereby depleting the AMP pool within the cell. This will shift the adenylate kinase reaction towards ATP production. Indeed, in rapidly dividing cancer cells there is an upregulation of PNC leading to a shift in the equilibrium of the adenylate kinase reaction towards ATP synthesis[79].

PNC is also known to be upregulated in the skeletal muscles during exercise[80,81]. The fumarate generated in this cycle anaplerotically feeds the TCA cycle and thereby contributes to the increased ATP requirement during exercise[82]. In IE stages of *P. falciparum*, fumarate is recycled to aspartate and pyruvate (Figure 3). Malate, the first product of fumarate metabolism, is seen to be secreted as waste as well as utilized by the parasite to generate OAA[4,42]. A recent report by Storm and Muller[83] has shown that knockout of PEP carboxylase (PEPC) (the reaction catalysed by PEPC is the reverse of PEPCK) is conditional to supplementing the medium with millimolar concentrations of malate. The mechanism by which the activity of PEPC becomes essential has been attributed to the production of OAA.

OAA forms a metabolic node from which other reactions can take place. One of the metabolites that can be formed from OAA is aspartate involving the enzyme aspartate aminotransferase (AAT). A recent study using *P. falciparum* AAT-specific peptide inhibitors has shown the essentiality of the enzyme activity for parasite growth[76]. The asparate formed can be used for protein and pyrimidine biosynthesis and this is evident by the incorporation of radioactivity in parasite protein and DNA fractions when incubated with [2,3-^{14}C fumarate]. This could also serve as a mechanism by which purine nucleotide metabolism regulates pyrimidine nucleotide synthesis in the parasite. Additionally, aspartate regeneration from fumarate (produced through PNC) is a likely to be important in *P. falciparum*, as amino acid biosynthesis is absent in the parasite. In cancer cells, increased flux through PNC results in a net anabolic contribution. Deamidation of glutamine to glutamate and subsequent formation of aspartate by aspartate aminotransferase has been reported in many forms of cancer. The aspartate thus formed fuels the PNC in these cells[84,85].

Further, OAA can also be converted to lactate by the sequential action of PEPCK, pyruvate kinase and lactate dehydrogenase. The net result of these reactions is the oxidation of one molecule of NADH to NAD^+ in the cytosol. The NAD^+ formed can fuel the step catalysed by glyceraldehyde 3-phosphate dehydrogenase in the glycolytic pathway and also feed into other pathways too.

Ammonia assimilation is suggested to be present in the parasite[32] and hence the ammonia generated through PNC can be used for such reactions. However, it should be noted that a considerable amount of ammonia is secreted as a metabolic waste by the parasite[86]. In yeast, ammonia is reported to have a regulatory role in glycolysis by acting as an allosteric activator of PFK[87,88] and the extent of its regulatory activities in *P. falciparum* is yet to be examined. The temporal separation of fumarate and ammonia-generating reactions of PNC has been suggested to be involved in pH regulation in the skeletal muscles of rainbow trout[89]. The exact nature of PNC in terms of temporal separation of its reactions and its role in pH maintenance is yet to be validated in the parasite.

Conclusion

Measurement of fluxes operating through interconnected pathways would provide a deeper understanding into the regulation of the pathways and the channelling of metabolites through them. The PNC plays a regulatory role in energy metabolism and maintenance of acid–base homeostasis. Due to the parasitic nature of *P. falciaparum*, the regulatory processes of its metabolic pathways may be different from those of free-living organisms. The individual fluxes of each of the reactions of PNC along with their temporal regulation across the different stages are yet to be examined. The essentiality of fumarate generated through PNC and the reaction intermediates it generates have to be examined by the generation of conditional knockouts of the genes of the cycle. Analysis of isotopomer distribution of labelled substrates using specific PNC knockout strains would permit evaluation of the fluxes operating through these pathways.

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