REVIEW ARTICLE

Survival strategies of the malarial parasite *Plasmodium falciparum*

T. N. C. Ramya*[†], Namita Surolia[†] and Avadhesha Surolia*[‡]

*Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India
[†]Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560 064, India

***Plasmodium falciparum*, the protozoan parasite causing falciparum malaria, is undoubtedly highly versatile when it comes to survival and defence strategies. Strategies adopted by the asexual blood stages of *Plasmodium* range from unique pathways of nutrient uptake to immune evasion strategies and multiple drug resistance. Studying the survival strategies of *Plasmodium* could help us envisage strategies of tackling one of the worst scourges of mankind.**

WHY would one want to study strategies adopted for survival by a parasite like *Plasmodium falciparum*? An analogy might help to appreciate the importance of survival strategies. A smart burglar studies his target house till he knows it like the back of his hand, before breaking into it. A successful parasite is somewhat like the smart burglar. It knows exactly what resources are available to it from the host, and accordingly adopts strategies either to modify the host or to make the most of it. Survival strategies are indispensable indeed, to the parasite, but why again would one want to study them? Extending the analogy a little further, to crack a tough case, a detective would need to put himself into the shoes of the burglar. We would do well, too, at controlling parasites and tackling the diseases they cause, if we were to emulate the smart detective!

Before going into the strategies adopted by it, it would be fruitful to have a look at the parasite. *Plasmodium* is a protozoan parasite classified under the phylum Apicomplexa which also includes parasites like *Toxoplasma*, *Eimeria* and *Cryptosporidium*, all of which are endowed with a specialized apical complex for host cell invasion. Four species of *Plasmodium* infect man – *P. vivax*, *P. ovale*, *P. malariae* and *P. falciparum*, among which *P. falciparum* is by far the most virulent[1]. Malaria, the disease caused by *Plasmodium* continues to be the disease with the highest mortality rate, next only to tuberculosis. It is endemic to around 100 countries in the world. Approximately 500 million cases of malaria are reported every year, and around 3000 children die of malaria every day[1]. Emerging resistance to all currently prescribed drugs limits treatment of malaria today. Hence, the identifica-

tion of antimalarial drug targets is of critical importance. Studying the survival strategies of *P. falciparum* is therefore extremely relevant.

A quick look at the lifecycle of this parasite (Figure 1) reveals a complex process involving two hosts – the vertebrate host, man, and the invertebrate host (vector), the female *Anopheles* mosquito[2]. Infection in man begins with the bite of a female *Anopheles* mosquito harbouring sporozoites in its salivary gland. The sporozoites in the blood stream find their way to the liver and invade the hepatocytes within 30 min of being released by the mosquito. Asexual reproduction (exoerythrocytic schizogony) in the liver releases merozoites, which are the first stage of the 48-h asexual reproduction cycle in the red blood cells (RBCs; erythrocytic schizogony). The blood stages constituting this cycle, studied by light and electron microscopy[3], are the merozoite, the ring, the trophozoite and the schizont which ruptures to release merozoites that invade fresh RBCs (Figure 1). The merozoite, which is the only stage to be briefly extracellular during invasion, and therefore of great immunological interest, is suitably armed with a bristly coat and an apical complex comprising the rhoptries, dense granules and micronemes (required for RBC invasion). The ring, so called because of its signet ring-like appearance in light microscopy, is an actively feeding stage. So also is the trophozoite that differs only by its larger size and by the presence of cytostomes for endocytosis of the host cell stroma. The schizont is the asexually dividing stage. Tangential mitotic divisions along with an increase in the number of organelles and the formation of apical complexes in the schizont result in the formation of a number of merozoites within the parasitophorous vacuole which envelops the parasite plasma membrane. Some of the merozoites that invade the RBCs do not go through the asexual reproduction cycle, but mature to male and female gametocytes which are picked up by the female *Anopheles* mosquito during a blood meal. Mature sporozoites released from the oocyst following fertilization of the gametes in the midgut of the mosquito and meiosis, find their way to the salivary glands and are injected into a human host when the mosquito takes a blood meal, and thus the cycle continues.

Survival strategies adopted by parasites vary with the habit of the parasite. An intracellular parasite has the

undoubtedly big advantage of not eliciting much of an immune response from the host, while the extracellular parasite scores over its intracellular counterpart with regard to tissue colonization and easy entry into and exit from a host. Common survival strategies adopted by parasites are those for attachment to host tissues, access to host nutrients and avoidance of the host defence system[4].

Strategies adopted by the asexual blood stages of *P. falciparum* include transport of macromolecules and ions across the RBC into the parasite – access to host nutrients; haemoglobin digestion and haem detoxification; novel metabolic pathways; immune evasion strategies and multiple drug resistance.

Among these, the former three are discussed in this review. Immune evasion strategies and multiple drug resistance have been the topics of several past reviews, and have not been covered here.

Transport of macromolecules and ions across the RBC into the parasite – access to host nutrients

P. falciparum is a rapidly growing parasite – every 48 h, 12 to 16 merozoites emerge out of a schizont. To fuel this phenomenal rate of growth, the parasite needs nutrients from outside the infected cell. The host RBC is metabolically inert and is not capable of transporting in sufficient quantity, at least some of the nutrients and ions which *P. falciparum* requires to satisfy its voracious appetite. The parasite would therefore need to increase the permeability of the red cell membrane to nutrients and ions. The permeability of infected red cell membranes could theoretically be increased by the presence of activated native transporters or parasite-inserted channels in the red cell membrane, or by an increase in membrane fluidity brought about by the insertion, in the membrane, of parasite-derived lipids or proteins which cause structural defects (Table 1). Evidence today points to two major types of trafficking pathways besides endocytosis of the host cell stroma into the parasite (Figure 2).
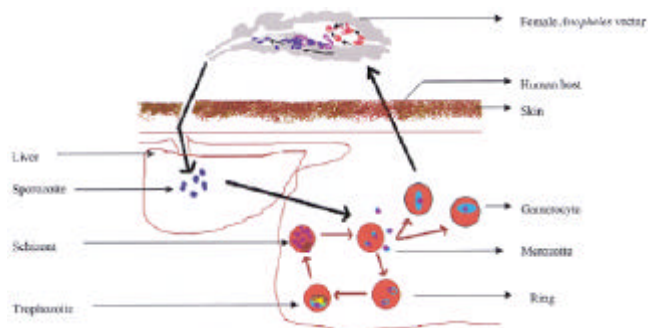

Figure 1. Life cycle of *Plasmodium falciparum* in man and the invertebrate vector, *Anopheles*, showing the sporozoites in the salivary glands of *Anopheles* and the asexual stages – merozoites, rings, trophozoites and schizonts, and the sexual stages – male and female gametocytes in the red blood cells of man.

Direct pathway

This model is supported by evidence in favour of the presence of an aqueous parasitophorous duct connecting the parasitophorous vacuole to the external medium. Serum macromolecules can therefore be accessed by the parasite by passive diffusion into the parasitophorous vacuole through the parasitophorous duct, followed by endocytosis into the parasite. Evidence in favour of this duct comes from fluorescence microscopy studies using fluorescence dextrans and immunoglobins[5]. Though these initial studies evoked considerable controversy[6,7], further evidence in the form of transmission electron microscopy has been reported recently[8]. Moreover, fragments of recombinant amino levulinic acid dehydratase added to the culture medium were shown to enter infected red cells and inhibit *P. falciparum* growth[9]. Given the absence of endocytosis and other mechanisms for the import of these macromolecules, it is possible that the duct indeed exists. The bulk of the evidence available today, however, tilts the balance in favour of the sequential pathway.

Sequential pathway

The sequential model involves the transport of solutes from the serum, first across the red cell membrane and

Table 1. Possible mechanisms to increase the permeability of the red cell membrane

Protein-mediated
- Activation of host transporters.
- Insertion of parasite-derived transporters in host plasma membrane.
- Leaks resulting from structural defects in host membranes with parasite-inserted proteins.

Lipid-mediated
- Increased fluidization caused by parasite-inserted lipids in host membrane.
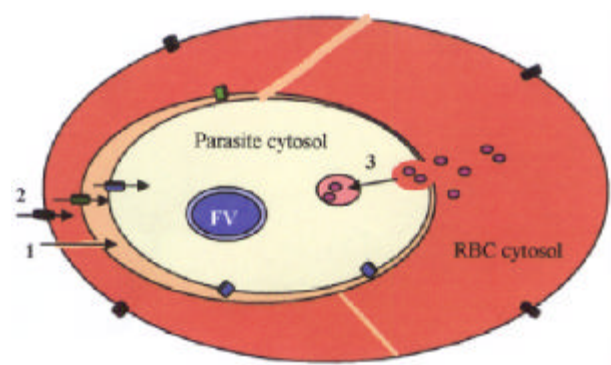

Figure 2. Two models for the uptake of nutrients and ions by *Plasmodium* – (1) direct pathway and (2) sequential pathway. The direct pathway makes use of the parasitophorous duct, which is continuous with the parasitophorous vacuole. The sequential pathway involves the passage of solutes sequentially through the protein channels on the red cell membrane (black), parasitophorous vacuolar membrane (green), and the parasite plasma membrane (blue). The endocytosis pathway (3) allows uptake into the food vacuole (FV) from the red cell cytosol.

then across the parasitophorous vacuolar membrane (Figure 2). This model is supported by evidence in favour of the presence of voltage-dependent, anion-selective channels (however whether of host or of parasite origin is not known as yet) on the membrane of infected red cells demonstrated by tracer studies, and recently by whole cell conductance measurements[10–12]. Voltage-dependent channels have also been shown to be present on the parasitophorous vacuolar membrane by patch clamp experiments[13,14]. Besides these components of the sequential pathway, a tubovesicular membrane network, which extends out of the parasitophorous vacuolar membrane, has been demonstrated by Bodipy ceramide membrane staining as well as by Lucifer yellow accumulation experiments in the parasite[15]. Lucifer yellow accumulation in the infected red cell was inhibited by PPMP, an inhibitor of the enzyme, sphingomyelin synthase, which is involved in the synthesis of the abundant sphingomyelin of the tubovesicular membrane network. The tubovesicular membrane network could allow the parasite efficient access to nutrients.

The solutes transported into the parasitophorous vacuole by either of these models are taken up by the parasite with the help of solute-specific transporters present on the parasite plasma membrane[10,16]. Transporters specific to several amino acids, nucleotides and glucose are present[17]. So also is a V-type ATPase that sets up a proton gradient allowing the inward transport of pantothenic acid and K^+, and the extrusion of lactate and glutamate across the plasma membrane[18,19]. Additionally, Na^+/H^+ and Ca^{2+}/H^+ ion exchangers are present[16].

Protein trafficking

How are the changes in the RBC transport properties brought about by *P. falciparum*? During the course of *P. falciparum* infection, the red cell membrane and cytosol as well as the parasitophorous vacuole are implanted with several proteins of parasite origin[20,21], which probably are responsible for these changes, besides playing a role in cytoadherence and vessel occlusion, the characteristic pathologic condition of cerebral malaria. How do these proteins find their way across the parasite plasma membrane and in the former case, across the parasitophorous vacuolar membrane too, to the red cell? In the absence of any endogenous transport machinery, export mechanisms must be engineered by the parasite. Two models, the one-step model and the two-step model, have been put forward to explain this enigma[22] (Figure 3).

According to the one-step model, the parasite cytosol has two types of transport-vesicles to traffic proteins to the parasitophorous vacuole and the red cell respectively. The first fuses with the parasite plasma membrane to release its protein contents into the parasitophorous vacuole, and the second fuses with regions of the parasite plasma membrane which are closely juxtaposed to the

parasitophorous vacuolar membrane, releasing its protein contents directly into the red cell cytosol[22]. However, no evidence for the presence of two types of vesicles has been reported so far.

According to the two-step model, all parasite proteins to be exported outside the parasite are transported by vesicles, which fuse with the parasite plasma membrane, releasing them into the parasitophorous vacuole. Proteins whose destination is beyond the parasitophorous vacuole pass through the parasitophorous vacuolar membrane presumably with the help of chaperones in the parasitophorous vacuole, which keep these proteins in the unfolded state. It is also possible that these proteins have very little conformation and hence can easily cross the membrane. Immunofluorescence microscopy and protease protection analyses done with parasite protein markers for each of the compartments of the infected red cell (aldolase for the parasite plasma membrane, SErine-Rich Protein (SERP) for the parasitophorous vacuole, and glycophorin-binding protein for the red cell cytosol) were in agreement with the two-step model[22,23].

Within the red cell cytosol itself, the temporal and spatial nature of protein targeting by the parasite as shown by localization experiments, suggests multiple pathways of protein delivery, unique targeting signals and regulation of secretory transport apparatus[24–26]. Though some parasite proteins which are exported do have ER (endoplasmic reticulum) signal sequences, and merozoite proteins that contain DBLs (Duffy Binding-Like) have canonical signal sequences for cotranslational ER recruitment, many exported proteins do not have an N-terminal hydrophobic ER signal sequence, and signal sequences for yet others such as the VARs which are targeted to the red cell membrane are yet to be elucidated[27,28]. GPI anchors, N-terminal transit peptide sequences and other motifs play a role in protein trafficking to various locations within the parasite itself[27].

With regard to the secretory transport apparatus, the ER has been indicated as part of the secretory pathway by electron microscopy as well as by experiments showing Brefeldin A-sensitivity of protein transport[29,30]. An unstacked Golgi lacking cisternae and glycosyl transferases and glycosidases, has been demonstrated by deconvolution microscopy and immunoelectron microscopy, as also by the existence of Golgi marker proteins, sphingomyelin synthase, PfERD2 and Rabs1, 6 and 11 (refs 29, 31 and 32). Besides these components of the secretory pathway within the parasite, plasmodial homologues of the COPII proteins, Sar1p and Sec31p, as well as the homologue of N-ethyl maleimide-sensitive factor (PfNSF), which are involved in secretory vesicle exit from ER and fusion of secretory vesicles with their target membranes respectively, have been localized to structures outside the parasite in the erythrocyte cytoplasm, suggesting that the malaria parasite exports components of its trafficking apparatus, thus establishing a vesicle-mediated traffick-

ing pathway outside the boundaries of its own plasma membrane[33–36].

Besides the parasite-derived components of the secretory pathway in the erythrocyte cytosol, the parasite also seems to recruit host machinery for the trafficking of its proteins. This is supported by studies on chaperones recruited during the transport of parasite-secreted cargo proteins to the erythrocyte plasma membrane[37]. None of the reported chaperones of parasite origin are present in the erythrocyte cytoplasm. The erythrocyte cytosol instead contains chaperones of the host (Hsp70, Hsp90, Hop60) which can be detected by hypotonic lysis and detergent solubilization experiments. Unlike their soluble nature in normal erythrocytes, host chaperones are recruited in membrane-bound, detergent-resistant complexes in infected cells, confirming their recruitment in the assembly of parasite proteins such as knob subunits (PfHRP1) at the erythrocyte surface.
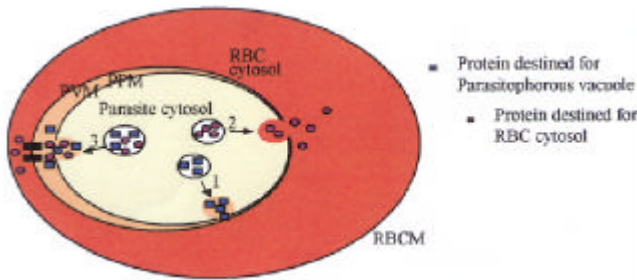

Figure 3. Two models for the trafficking of parasite proteins to the red cell cytosol and the parasitophorous vacuole. In the one-step-model, parasite proteins destined for the parasitophorous vacuole and the RBC cytosol are transported by separate vesicles (1, 2). In the two-step-model, (3), both classes of proteins are transported by vesicles into the parasitophorous vacuole. Proteins destined for the red cell cytosol are then transported via a second step through channels on the parasitophorous vacuolar membrane (PVM). PPM, Parasite plasma membrane; RBCM, Red blood cell membrane.

Besides host chaperones, there is also definitive evidence that despite the absence of endocytosis, transmembrane proteins in the host red cell membrane are recruited by the parasite and imported to the parasitophorous vacuolar membrane[38]. These are, again, mainly protein components of detergent-resistant membrane rafts in red cell membrane and are detected in rafts in the parasitophorous vacuolar membrane. Disruption of either erythrocyte or vacuolar rafts is detrimental to infection, suggesting that raft proteins and lipids are essential for the parasitization of the red cell.

Haemoglobin digestion and haem detoxification

Haemoglobin constitutes more than 90% of the total protein in a red cell. At 20% parasitaemia, during the trophozoite stage, more than 60% of the host haemoglobin, approximately 140 g, in a red cell is digested by the parasite. Besides amino acid uptake and *de novo* amino acid synthesis in the parasite, haemoglobin digestion is a major source of amino acids (Figure 4). Haemoglobin is taken up by simple endocytosis of the host cell stroma during the ring stage of the parasite and with the help of specialized organelles known as cytostomes during the trophozoite stage. The endocytotic vesicles empty their contents into the acidic food vacuole, the specialized lysosome-like organelle of the parasite. The aspartic protease, plasmepsin I, initiates haemoglobin digestion by cleaving the highly conserved haemoglobin hinge region between the Phe33 and the Leu34 amino acids. This cleavage destabilizes the globin molecule, making it susceptible to further enzymatic action[39]. A recently discovered aspartic proteinase, plasmepsin IV from the human malaria parasite *P. falciparum* is also able to hydrolyse human haemoglobin at a site known to be the essential primary cleavage site in the haemoglobin degradation pathway[40].
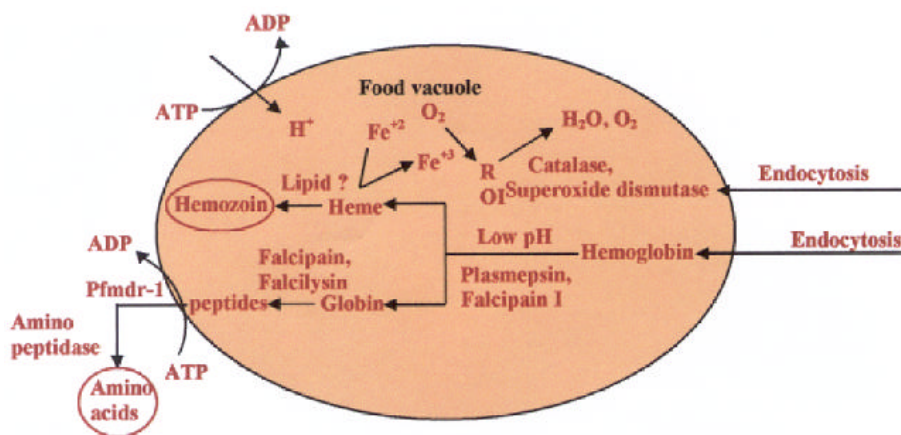

Figure 4. Haemoglobin digestion and haem metabolism in the food vacuole of *Plasmodium*. Endocytosed host haemoglobin is a source of amino acids for the parasite. Toxic haem is polymerized to hemozoin within the food vacuole and reactive oxygen intermediates generated by oxidation of haem are detoxified by catalase and superoxide dismutase.

Three papain-family cysteine protease sequences have been identified in the *P. falciparum* genome, but the specific roles of their gene products and other plasmodial proteases in haemoglobin hydrolysis are uncertain[41]. Falcipain 1, like plasmepsin, is capable of cleaving native haemoglobin, while falcipain 2 cleaves denatured haemoglobin. Falcipain 2 and falcipain 3 are principally expressed by early trophozoites and appear to be located within the food vacuole, the site of haemoglobin hydrolysis. Both proteases require a reducing environment and acidic pH for optimal activity, and both prefer peptide substrates with leucine at the P(2) position. The proteases differ, however, in that falcipain 3 undergoes efficient processing to an active form only at acidic pH, is more active and stable at acidic pH, and has much lower specific activity against typical papain-family peptide substrates, but has greater activity against native haemoglobin. Thus, falcipain 3 is a second *P. falciparum* haemoglobinase that is particularly suited for the hydrolysis of native haemoglobin in the acidic food vacuole[42].

Falcilysin cleaves polypeptides formed by the action of plasmepsin and falcipain[43]. The peptides formed by the action of all these enzymes are presumably transported to the parasite cytosol by the transporter Pfmdr-1, where they are digested by aminopeptidases[44,45]. Free haem spells potential disaster for membranes, and besides, the oxidation of Fe^{2+} to Fe^{3+} generates an electron, which results in the release of free radicals like superoxide and H_2O_2. It is therefore imperative that haem is converted to a less toxic compound. Previous findings suggested that haem is converted to insoluble haemozoin (***b***-haematin) by a polymerization reaction[46]. Chemical analysis has shown that *P. falciparum* trophozoites contain approximately 61% of the iron within parasitized erythrocytes, 92% of which is located within the food vacuole. ^{57}Fe-Mössbauer spectroscopy and electron spectroscopic imaging show that haemozoin is the only detectable iron species in trophozoites[47].

The polymerization to haemozoin is not a spontaneous reaction[48], and neither is it an enzyme-catalysed reaction as considered earlier, since the reaction proceeds in the presence of proteases and at very high temperatures[49,50]. Evidence suggests that the polymerization is probably a physio-chemical, lipid-catalysed reaction in the infected cells[51–54]. Malaria parasite homogenate, lipid extracts, and an unsaturated fatty acid, linoleic acid have been shown to promote beta-haematin formation *in vitro*. The presence of ascorbic acid, reduced glutathione, sodium dithionite, beta-mercaptoethanol, dithiothreitol and superoxide dismutase, as also peroxidized lipid preparations and oxygen depletion in the reaction mixtures inhibit the lipid-catalysed beta-haematin formation. ***b***-free-radical chain reaction breakers such as *p*-aminophenol also inhibit haematin formation. All this calls for an oxidative mechanism of lipid-mediated ***b***-haematin formation,

which may be mediated by the generation of some free-radical intermediates of haem.

Histidine-rich proteins are thought to play a role in polymerization[55]. Histidine-rich protein-2 (PfHRP2) not only binds up to 50 molecules of ferriprotoporphyrin (FePPIX), it also modulates the redox activity of FePPIX, imparting to the PfHRP2–FePPIX complex previously unrecognized antioxidant properties[56]. The free radicals generated from haem are taken care of by endocytosed host superoxide dismutase and, by catalase and glutathione reductase, which are possibly of parasite origin[57].

Novel metabolic pathways in *P. falciparum*

Metabolic pathways in a parasite, which often are the key to discovering peculiarities and idiosyncrasies that can be exploited in drug design, are intimately related to the niche inhabited by the parasite.

The main source of energy for the rapidly growing *P. falciparum* is the glycolysis pathway[58]. The rate of energy generated by lactate fermentation following glycolysis can be as much as 100-fold compared to what can be obtained on oxidative phosphorylation. The parasite has, thus, done away with the citric acid cycle, and the pyruvate generated by glycolysis is converted to lactate, which is the end product of glycolysis in the parasite. Moreover, unlike the host enzyme, there is no substrate inhibition of the enzyme LDH by pyruvate, facilitated perhaps by an insertion of five amino acids, which brings about a shift in the binding cleft of NADH to LDH observed in the crystal structure of the parasite LDH enzyme. This allows fast energy production to fuel the rapid rate of growth of the parasite inside the red cell. Enzymes of the glycolysis pathway in *P. falciparum*, in particular, triose phosphate isomerase, have been studied in great detail, and offer promise of therapeutic leads and insights into antimalarial drug design[59,60].

Reactive oxygen species (ROS) are a nightmare for any intracellular pathogen. The malarial parasite, however, has its own battery of defence tactics lined up against ROS. The parasite has its own hexose monophosphate shunt pathway, including the first enzyme G6PD, and glutamate dehydrogenase that generates NADPH, which it uses along with host reducing power to ward-off damage from the ROS generated by itself, the RBC as well as host immune cells[61]. ROS are generated in particular during oxidation of Fe^{2+} in haem to Fe^{3+}. Superoxide generated during this process is dismutased to H_2O_2 by SOD, and H_2O_2 is reduced by catalase and glutathione peroxidase. The oxidized glutathione formed during this process is reduced back to its original state by glutathione reductase at the expense of NADPH oxidation. All these enzymes, i.e. SOD, catalase, glutathione peroxidase and glutathione reductase are produced by the parasite. The parasite SOD shows Mn/Fe SOD activities,

unlike the host Cu/Zn SOD that incidentally is also endocytosed by the parasite, and is regarded a potential target for antimalarial chemotherapeutics. The glutathione reductase and thioredoxin reductase systems present two powerful ways of combating oxidative stress in the parasite[62]. GSH, which is the major low-molecular-mass tripeptide thiol in most organisms, maintains a reduced intracellular environment and protects cellular components from the damaging oxidation. GSH is synthesized by the action of two ATP-dependent enzymatic steps, in which gamma-glutamyl cysteine synthetase (gamma-GCS) catalyses the ligation of glutamate and cysteine, and subsequently glutathione synthetase (GS) adds glycine to the dipeptide. The synthesis of gamma-glutamyl cysteine has been demonstrated in the parasite using the specific gamma-GCS inhibitor, buthionine sulphoximine[63]. Genes for glutathione reductase (Pfgr1) and thioredoxin reductase (Pfgr2) have been identified. Thioredoxin reductase (TrxR) knockouts in *P. falciparum* are not viable, suggesting that TrxR is essential for *P. falciparum* erythrocytic stages[64].

Nucleic acid metabolism is one area where differences exist between the pathways of *P. falciparum* and those of the human host. *P. falciparum* synthesizes purines by the salvage pathway[65] and pyrimidines *de novo*, the reverse of the predominantly used pathways in the host cell (mammalian cells synthesize purines *de novo* and either salvage or synthesize pyrimidines *de novo*), and are therefore of considerable interest from a pharmacological point of view. The purine salvage pathway utilizes the plasmodial enzyme, HGPRT, adenyl succinate synthase and inosine dehydrogenase[58,66]. HGPRT catalyses the transfer of a phosphoribosyl moiety from hypoxanthine to inosinic acid. The low activity of this enzyme recovered from cell lysates as well as the considerable homology with its human counterpart, make it an improbable drug target. The predominant precursor for salvage is hypoxanthine, formed during ATP catabolism. However, inosine formed from adenosine by adenosine deaminase or taken up from the host could also be the precursor. Both AMP and GMP can be formed from IMP by adenyl succinate synthase and adenyl succinate lyase, and IMP dehydrogenase and GMP synthase respectively.

The enzymes, carbomoyl phosphate synthase, aspartate transcarbamylase, dihydroorotase, dihydoorotate dehydrogenase, orotate phosphoribosyl transferase, and orotidine 5′-phosphate decarboxylase bring about pyrimidine synthesis *de novo*. The pathway is similar to that in other eukaryotes, except that unlike in other eukaryotes, the first three enzymes are separate from one another[58]. The mitochondrial enzyme, dihydroorotate dehydrogenase, catalysing the single redox reaction in the pyrimidine *de novo* synthetic pathway, links electron transport in the mitochondrion with pyrimidine synthesis and is also the major supply of electrons for the mitochondrial electron transport chain. Dihydroorotate-dependent respiration is facilitated by the complex II (succinate-ubiquinone reductase/quinol-fumarate reductase) in the electron transport chain, which functions as a quinol-fumarate reductase[58].

Folate, which is required for purine salvage and *de novo* biosynthesis of amino acids and pyrimidines, is synthesized either by the salvage or the *de novo* pathway in the parasite, unlike the host, which can only salvage folates[67].

While all these metabolic pathways do offer a few drug targets, the presence of an equivalent enzyme in the host hampers the development of a good antimalarial without any effect on the host enzyme. The discovery of the green-algal-plastid-like organelle, the apicoplast in the parasite, however, offers new hope[68]. Going by similarity, it is expected that the plastid be involved in biosynthesis pathways such as those of fatty acids, aromatic amino acids and haem, and in fact, the type II fatty acid biosynthesis pathway[69] and the haem biosynthesis pathway[70] do exist in the parasite.

The fatty acid biosynthesis pathway in the parasite is of great pharmacological interest, thanks to the host fatty acid biosynthesis pathway being of type I[69], unlike that of the parasite which has a fatty acid synthase II system in which the subsequent reactions of the pathway are catalysed by independent enzymes. This difference could be used in designing drugs against each of the independent enzyme targets of the pathway. The metabolic pathway involves the enzymes malonyl transacetylase (Fab D), *b*-ketoacyl ACP synthase (Fab H, B/F), *b*-ketoacyl ACP reductase (Fab G), *b*-hydroxy acyl ACP dehydrase (Fab A/Z) and enoyl ACP reductase (Fab I). The inhibitors of the enzymes, enoyl ACP reductase and *b*-ketoacyl ACP synthase, i.e. triclosan and cerulenin are effective in killing the parasite *in vitro* as well as *in vivo* in *P. berghei*. Triclosan inhibition of *b*-enoyl ACP reductase has also been extensively characterized and promises to be an excellent antimalarial target[71–73].

P. falciparum is engaged in biosynthesis of glycerolipids during its blood stages. An aquaglyceroporin (PfAQP) has been implicated in the transport of sugar alcohols and as expected, is expressed in blood-stage parasites throughout the development from rings through schizonts. It is also presumed to function in the protection from osmotic stress and in access to the serum glycerol pool for ATP generation[74].

It does seem extremely paradoxical that living as it does in a sea of haemoglobin, *Plasmodium* would have its own haem biosynthesis pathway. The toxic nature of haem and the reactive oxygen intermediates released during its oxidation, however, warrant the conversion of haem to the non-toxic haemozoin as mentioned earlier, thus making it imperative for *Plasmodium* to synthesize its own haem. Haem is required for incorporation into cytochromes and other haem-containing proteins, as well as for the reticulocyte lysate-like haem-dependent protein

synthesis occurring in the parasite. Evidence for *de novo* haem synthesis in *P. falciparum* was reported by Surolia and Padmanaban[70] who demonstrated that the precursors for ALA are glycine and succinate but not glutamate, as in animals, fungi and proteobacteria and unlike most bacteria and plants. Wilson *et al.*[75] later identified a potential ALAS and suggested that the malarial enzyme ALAS is a mitochondrial protein like human ALAS. The genome sequencing projects for several species of *Plasmodium* suggest that malaria parasites may have a complete set of genes for haem biosynthesis. Bonday *et al.*[76] and Padmanaban and Rangarajan[77] purified the second enzyme of the pathway, ALAD. Their immunolocalization experiments led them to conclude, however, that the murine malarial parasite imports the host ALAD for haem biosynthesis. Recently, however, ALAD cDNA has been cloned and is shown to be functional by a complementation assay[78]. Sequence alignments suggest that the enzyme is closely related to the Zn^{2+}-independent enzymes of plants. The gene further has a putative N-terminal plastid-targeting sequence. This finding implicates a role for the observed contact between the two organelles in electron microscopy, the mitochondrion and the plastid, as being required for the transport of ALA from the first enzyme ALAS in the mitochondrion to the second enzyme ALAD in the plastid. With other enzymes of the pathway still to be studied, one cannot completely rule out the possibility of *Plasmodium* importing host enzymes for haem synthesis.

The shikimate pathway, essential for the production of a plethora of aromatic compounds in bacteria, fungi and plants, also exists in apicomplexans, including *P. falciparum*[79]. Seven enzymes of the shikimate pathway catalyse sequential conversion of erythrose-4-phosphate and phosphoenol pyruvate to chorismate. Chorismate is the substrate for subsequent reactions, which culminate in the production of folates, ubiquinone, naphthoquinones and the aromatic amino acids, tryptophan, tyrosine and phenylalanine. The presence of the shikimate pathway explains the efficacy of the herbicide glyphosate, in preventing the growth of the parasites.

The *P. falciparum* apicoplast genome, measuring just about 35 kb, is much smaller than its plastid ancestor. This is mainly due to the transfer of the bulk of the plastid genes to the nuclear genome, and the consequent transport of their encoded proteins to the plastid[80]. Nuclear encoded proteins headed towards the apicoplast are guided by their bipartite N-terminal apicoplast-targeting signals, containing a signal peptide that targets proteins to the secretory pathway, and an NKI-rich transit peptide with a net positive charge, that targets the protein across the multiple membranes of the apicoplast that is cleaved off by a stromal-processing peptidase homologue after import of the protein[81–83].

Retention of the relic genome of the plastid and its as yet ill-defined involvement in protein synthesis might be linked to an important housekeeping process, i.e. guarding the type II fatty acid biosynthetic pathway from oxidative damage. The parasite plastid does not divide by constriction like in typical plants, and plastid-less parasites fail to thrive after invading a new cell, demonstrating that the existence of the plastid is obligatory in the parasite[84].

Recent years have thus seen the discovery and detailed elucidation of several metabolic pathways in *Plasmodium*. Getting to know the ins and outs of the infected red cell in the light of survival strategies adopted by *P. falciparum* should help us envisage ways of bringing under control, one of mankind's greatest scourges.

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