

# Of malaria, metabolism and membrane transport

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**With the sequencing of the *Plasmodium falciparum* genome now complete, increasing attention is turning to the function of gene products and to cell-regulatory processes. The combination of *in silico* analyses with modern molecular and biophysical methods is leading to rapid advances in our understanding of the mechanisms underlying the biochemistry and physiology of the parasite and its host cell. In this brief review, we present a 'snap shot' of recent work in this area, with particular emphasis on aspects relevant to the development of new antimalarial drugs.**

The first half of this article focuses on metabolic pathways and the enzymes involved. We initially consider the biochemical pathways present in the parasite apicoplast and mitochondrion. We present an overview of recent work on the function and three-dimensional structures of enzymes from *Plasmodium* spp., and consider, in particular, proteins involved in biosynthesis and redox metabolism. The second half of the article focuses on membrane transport, dealing first with ongoing controversies about the number and nature of novel channels underlying the enhanced transport of ions and metabolites across the erythrocyte membrane. We discuss recent work on ion homeostasis and Ca<sup>2+</sup> signalling in the intracellular parasite, and recent findings relating to transporter proteins involved in antimalarial drug resistance.

## Apicoplast pathways

The apicoplast, a relict chloroplast, has emerged as a promising target for new antimalarial drugs. The apicoplast synthesizes 23 proteins but is also estimated to import over 500 nuclear-encoded proteins, representing ~10% of the proteins encoded by the parasite genome [1]. The targeting sequence of the imported proteins can now be predicted with high accuracy and a role has been demonstrated for the 70 kDa heat-shock protein (HSP-70) in trafficking proteins to the apicoplast of *Plasmodium falciparum* [2].

The apicoplast proteins comprise complete pathways for fatty acid and non-mevalonate isoprenoid biosynthesis [3,4], as well as a partial set of haem synthesis enzymes. The second enzyme of the haem biosynthesis pathway of *P. falciparum*, delta-aminolevulinic acid dehydratase (ALAD),

has recently been localized to the apicoplast and characterized in detail. This protein represents only 10% of the total ALAD activity in the parasite, with the rest being attributed to host enzyme imported by the parasite [5].

Another apicoplast enzyme, ferredoxin-NADP<sup>+</sup>-reductase, and its redox partner, ferredoxin, form part of another interesting organelle-specific biosynthetic pathway, namely [Fe-S] cluster biosynthesis [6]. *Plasmodium* contains only one pyruvate dehydrogenase complex, located in the apicoplast, as well as one  $\alpha$ -ketoglutarate dehydrogenase complex and one branched-chain  $\alpha$ -keto-acid dehydrogenase complex, both in the mitochondrion. This indicates that pyruvate is converted to acetyl-coenzyme A (CoA) in the apicoplast, possibly for use in fatty acid biosynthesis, whereas the source of acetyl-CoA in mitochondria remains enigmatic [2]. Several anabolic pathways of the parasite apicoplast are fundamentally different from those in the human host. Many are likely to be essential to the parasite, and the proteins involved are therefore potential drug targets [6,7].

## Mitochondrial pathways

Asexual stages of malaria parasites contain a single mitochondrion that is often branched throughout the cell; by contrast, gametocytes can have several mitochondria [8]. By constructing green fluorescent protein (GFP) fusion proteins and developing a neural net-based computer application called PlasMit (<http://gecco.org.chemie.uni-frankfurt.de>), Bender *et al.* have identified 381 *P. falciparum* proteins likely to be located in the mitochondrion [8]. These proteins constitute an estimated 7.1% of the total proteins encoded by the *P. falciparum* genome, and include enzymes involved in electron transport, the citric acid cycle and ubiquinone biosynthesis. A functional respiratory chain, as well as the presence of an alternative NADH-quinone oxidoreductase and a malate-quinone oxidoreductase, has been demonstrated in *Plasmodium yoelii yoelii* [9]. In *P. falciparum*, a functional isocitrate dehydrogenase [10], as well as a GTP-AMP phosphotransferase [11], are likely to be localized to the mitochondrion and have been characterized biochemically. The unique properties of the respiratory chain in *P. falciparum* mitochondria have been reviewed by Mi-Ichi *et al.* [12], and a recent review by Vaidya [13] highlights the potential of both mitochondrial and apicoplast pathways as antimalarial drug targets.

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## Structural and functional studies of biosynthetic enzymes

Parasite-specific metabolic pathways are of obvious interest from the point of view of chemotherapy, and so too are differences in the three-dimensional structures or catalytic mechanisms of isofunctional parasite and host enzymes.

Structural studies of a range of *Plasmodium* enzymes are under way. Among the proteins being studied is *Plasmodium* dihydrofolate reductase-thymidylate synthase (DHFR-TS), a bifunctional enzyme of the folate pathway and a validated target for antimalarials. The structures of wild-type and 'drug-resistant' forms of *P. falciparum* DHFR-TS (PfDHFR-TS), both as the free enzyme and as the enzyme–drug complex, have been solved by X-ray crystallography. These studies have revealed the structural basis of drug resistance resulting from mutations around the active site. This information has been used to design new inhibitors with high affinity against both wild-type and mutant enzymes [14,15]. The structure of *Plasmodium vivax* DHFR (PvDHFR) was determined by homology modelling and molecular dynamics refinement. The comparison of the complexes of the antifolate inhibitor pyrimethamine bound at the active sites of PvDHFR and PfDHFR illustrates the possibility of using structure-based drug design to develop inhibitors that are effective against both plasmodial enzymes [16].

Polyamine biosynthesis in *P. falciparum* is regulated by a single, hinge-linked bifunctional enzyme, AdoMetDC/ODC (*S*-adenosylmethionine decarboxylase/ornithine decarboxylase). The bifunctional nature of this protein is unique to plasmodia. Furthermore, the protein contains parasite-specific inserts; recent data indicate that an interference with these parasite-specific regions of the protein might reduce essential protein–protein interactions and therefore represent an interesting starting point for the design of selective inhibitors [17]. The crystal structure of *P. falciparum* purine nucleoside phosphorylase (PfPNP) in complex with immucillin-H was solved recently, and provides a structural and mechanistic basis for the design of malaria-specific transition-state analogue inhibitors. The catalytic features of PfPNP indicate a dual function for this enzyme in purine salvage and polyamine metabolism [18]. Targeting enzymes involved in spermidine metabolism might represent a possible new antimalarial strategy [19].

Hydroxymethyl dihydropterin pyrophosphokinase (PPPK) and dihydropteroate synthase (DHPS) are obligatory enzymes in *de novo* folate biosynthesis in protozoan parasites. In *P. falciparum*, a bifunctional PPPK-DHPS is formed from a single polypeptide. Mutations in DHPS have been linked to resistance to the antimalarial drug sulfadoxine, which competes with *p*-aminobenzoate. The role of these mutations has been studied by expressing six relevant alleles of the gene in DHPS-disrupted *Escherichia coli* in the presence of different drugs. The results might enable the development of novel chemotherapeutic agents [20]. The full-length *P. falciparum* *ppdk-dhps* gene was recently expressed in *E. coli* and the homotetrameric protein was purified for crystallization and drug screening [21]. The effect of modulation of DHPS activity on parasite

phenotypes has been studied using a gene-disruption protocol that was recently developed for studying folate metabolism in *P. falciparum* [22]. The data indicate that DHPS activity above a low but critical level is essential, regardless of the availability of salvageable folate. However, there was no evidence of a significant role for DHPS in folate salvage [23].

Pantothenate, the precursor of CoA, is taken up by the intracellular parasite by a H<sup>+</sup>-coupled transporter that is distinct from the Na<sup>+</sup>-coupled pantothenate transporters of mammalian cells [24]. The biosynthesis of CoA involves a series of five enzyme-mediated steps. The activity of dephospho-CoA kinase, the fifth enzyme of the pathway, has recently been detected in *P. falciparum*, consistent with the presence of the pathway in the parasite and raising the possibility of targeting this pathway with pantothenate analogues [25].

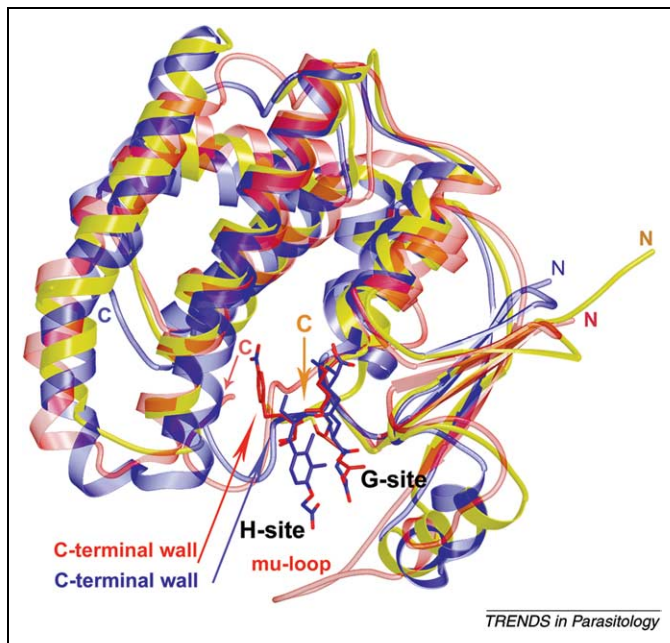
## Redox metabolism

Functional thioredoxin and glutathione systems have been demonstrated to contribute to antioxidant defence in *P. falciparum*, and both are considered attractive drug targets [26–28]. Thioredoxin reductase (a validated drug target), thioredoxins, glutaredoxins, and thioredoxin peroxidases have been characterized biochemically. A novel 22 kDa redox-active protein, plasmoredoxin (Plrx), is found exclusively in malaria parasites and is highly conserved between species. It is a member of the thioredoxin superfamily, but clusters separately from other members in a phylogenetic tree. Plrx offers the opportunity to improve diagnostic tools based on PCR or immunological reactions and might represent a suitable target for antimalarial drug development [29].

The crystal structure of *P. falciparum* glutathione *S*-transferase (GST) – the only GST isoenzyme in the parasite – has been solved at high resolution. The protein cannot be assigned to any known GST class and possesses a more solvent-accessible substrate-binding site than its human counterparts, which is a feature that might be exploited for the design of specific PfGST inhibitors [30]. The differences between the substrate-binding site (H-site) of a human mu-class GST and PfGST are illustrated in Figure 1.

The structure [31] and mechanism of *P. falciparum* glutathione reductase (GR) has also been elucidated. Three major features distinguish PfGR from human GR: the presence of three major peptide insertions, the sequence of the interface helices, and the intersubunit cavity (which differs in size, shape and chemical characteristics). PfGR is being studied as a target of the antimalarial agent methylene blue [32] and of double-headed prodrugs. The glyoxalase system, which detoxifies methylglyoxal, is another glutathione-dependent pathway that is presently under investigation in malaria parasites. The parasite system shows some unusual features (e.g. the subunit fusion of *P. falciparum* glyoxalase I [33]), encouraging the hope that it might be a suitable drug target.

A method for the stage-specific expression of genes contributing to antioxidant defence in *P. falciparum* microcultures has recently been established [34]. Campanale *et al.* studied the influence of ferriprotoporphyrin IX (FP)



**Figure 1.** Structural comparison of glutathione *S*-transferase enzymes. Superimposed structures of *Plasmodium falciparum* glutathione *S*-transferase (PfGST) (yellow and green), mu-class GST (red, 1c72) and pi-class GST (blue, 11 gs). The active site is composed of two binding sites: the G-site, which binds reduced glutathione, and the more-variable H-site. The mu-class enzyme contains the glutathione conjugate 1-hydroxy-2-S-glutathionyl-3-para-nitrophenoxy-propane (red ball and stick), and the pi-class enzyme contains the glutathione conjugate of ethacrynic acid (blue ball and stick). All three enzymes exhibit a similar binding mode for glutathione, but the conjugated moiety of glutathione stretches out into different directions of the H-site. A typical feature of mu- and pi-class enzymes is the C-terminal wall that lines the H-site. This feature is lacking in PfGST, resulting in a more solvent-accessible H-site. The figure was kindly provided by Karin Fritz-Wolf (Max-Planck Institute for Medical Research, Heidelberg, Germany).

on the activity of *P. falciparum* redox-active enzymes, as well as on glyceraldehyde phosphate dehydrogenase [35]. The results indicate that FP is likely to inhibit glycolysis, thus increasing the flux through the pentose phosphate pathway, which is the major source of NADPH. Blood stages of *P. falciparum* rely on glycolysis, coupled with homolactic fermentation, for ATP production. Heterocyclic,azole-based compounds have been identified as selective inhibitors of the lactate dehydrogenase (LDH) of the parasite, which plays a crucial role in NAD<sup>+</sup> regeneration. The compounds display activity against resistant parasite strains *in vitro*, and against *Plasmodium berghei* in BALB/c mice [36].

### Channels in the host erythrocyte membrane

It has been known for decades that, following infection by the malaria parasite, there is a profound increase in the permeability of the host erythrocyte membrane to a wide range of low-molecular-weight solutes. This increase is owing to the appearance in the host cell membrane, some 12–16 h after invasion, of ‘new permeability pathways’ (NPPs). These pathways are thought to provide the major route of entry of at least some essential nutrients required by the parasite (e.g. pantothenate [37]), and they mediate the efflux from the infected cell of various metabolic wastes, such as lactic acid, and amino acids originating from the digestion of haemoglobin. They are therefore of as significant interest as antimalarial drug targets.

The transport properties of the NPPs have been characterized in some detail (e.g. see Refs [38,39]). The pathways are broadly anion selective, but with a significant permeability to both organic and inorganic cations. They are inhibited by a range of classical anion transport blockers, and various derivatives thereof, some of which are effective at nanomolar concentrations (e.g. see Ref. [40]). Applying Occam’s Razor (‘one should not increase, beyond what is necessary, the number of entities required to explain anything’) to the results of experiments comparing the effects of different inhibitors on the transport of a range of different substrates led to the proposal that the NPPs represent a single class of anion-selective channels [39]. In the first reported ‘patch-clamp’ study of parasitized erythrocytes, Desai and colleagues observed a single class of ‘inwardly rectifying’ (IR) anion channels (i.e. channels that pass current into the cell more readily than they do out of the cell) present in infected but not uninfected erythrocytes that showed similar pharmacological and ion selectivity properties to the NPPs [41]. Similar, although not identical, data were obtained by Egée et al. [42]. However, Lang and colleagues have reported the presence in parasitized erythrocytes of three distinct ‘conductances’, which they have attributed to three discrete channel types: an IR anion channel, an ‘outwardly rectifying’ (OR) anion channel and a non-selective cation channel [43,44]. The group attributed the enhanced transport of organic solutes across the infected erythrocyte membrane to the OR channel [45].

In a recent collaborative study involving three groups, it was shown that the observation of the OR conductance was increased several fold in the presence of trace amounts of serum in the extracellular medium [46]. This might explain at least some of the differences between observations made in several different laboratories. Nevertheless, there remains active debate over the question of the number of channel types comprising the NPPs [45,47–49] (Figure 2) and over whether they originate from the host or the parasite. Several groups report observing similar conductances in uninfected erythrocytes exposed to particular stresses or stimuli [42,43], prompting the hypothesis that the channels underlying the NPPs are host erythrocyte proteins, activated by the parasite. Other groups have been unable to repeat these observations [50]. Desai and colleagues have reported intriguing strain-specific variations in the electrophysiological properties of the IR channel that they described in infected cells, and have interpreted the data as evidence that the channel is encoded by the parasite, rather than by the host [50]. Verloo *et al.* [51] have recently reported: (i) that, whereas *P. falciparum*-infected erythrocytes from normal donors show a prominent IR anion current, those from patients with cystic fibrosis do not; and (ii) that infected erythrocytes from normal and cystic fibrosis donors show the same parasite-induced uptake of at least some low-molecular-weight organic solutes [51]. The implication here is that the IR channel observed in the patch-clamp experiments might be a host protein, somehow associated with the cystic fibrosis protein (CFTR), and that, furthermore, it is not the pathway underlying the increased transport of solutes such as

amino acids across the infected erythrocyte membrane. This is perhaps consistent with the view that it is the more elusive (serum-dependent) OR conductance that is the electrophysiological manifestation of the NPPs [49]. However, the findings of Verloo *et al.* have been disputed by Desai and colleagues who have reported that *P. falciparum*-infected erythrocytes from donors with cystic fibrosis show the same inwardly rectifying  $\text{Cl}^-$  conductance as is seen in parasitized cells from normal donors [50] (Figure 2).

The sooner the proteins underlying the enhanced permeability of the parasitized erythrocyte are identified, the sooner the current controversies will be resolved. But this is proving far from straightforward, particularly in light of the present uncertainty as to their genetic origin.

### Transport of ions and metabolites in the intraerythrocytic parasite

In the parasite plasma membrane, a vacuolar-type  $\text{H}^+$  pump extrudes  $\text{H}^+$  [52], thereby generating a significant  $\text{H}^+$  concentration gradient [53] and a membrane potential of around  $-95$  mV [54]. Several different metabolite transporters have been characterized in the parasite, and several others have been expressed and characterized in *Xenopus* oocytes (reviewed by Ref. [55]). Two recent papers [56,57] report the characterization in isolated parasites of the parasite plasma membrane choline transporter, a saturable electrogenic carrier postulated previously [58] to be the site of action of a series of potent antimalarial choline analogues and shown in one of the two recent studies to be the route of uptake of anti-*Plasmodium* bis-amidine and bis-quaternary ammonium compounds into the parasite [56]. There is evidence for a  $\text{K}^+$  channel in the parasite plasma membrane [54] and one of at least two candidate  $\text{K}^+$  channels in the genome has recently been

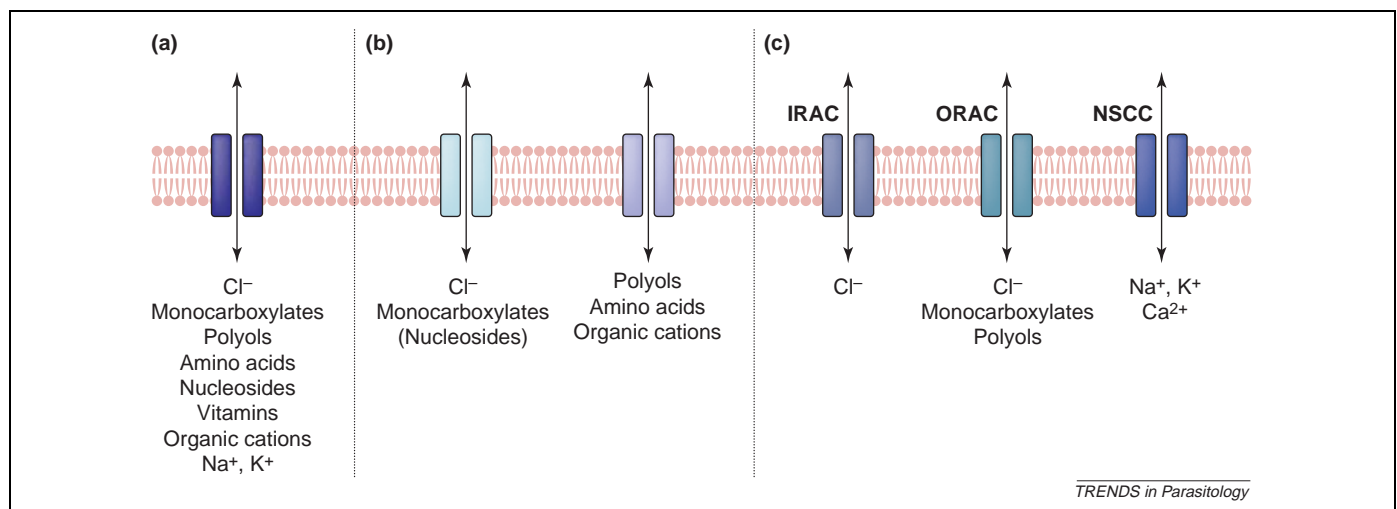
cloned [59], but not yet characterized. A parasite-encoded hexose transporter (PfHT) has been expressed in *Xenopus* oocytes (reviewed in Ref. [55]) and shown to be inhibited by a novel hexose derivative that inhibits parasite proliferation, both *in vitro* and *in vivo* [60]. In isolated parasites, the compound blocks the uptake of hexose sugars by the parasite, leading to a rapid decline in ATP levels and a loss of pH control [61].

In the initial *P. falciparum* genome annotation [62], relatively few transporters, and no channels, were identified. An approach based on analysing predicted protein hydrophathy plots has taken the number of candidate transporters in the genome to over 70 [63]. Ascribing function to these proteins presents a significant challenge, and heterologous expression of these proteins is, with just a few exceptions, proving difficult.

Within the parasite, there are several metabolite transporters predicted to be present on the apicoplast [1], and several recent physiological studies have revealed the presence on the digestive vacuole of the parasite of two discrete  $\text{H}^+$  pumps [64] and a  $\text{Ca}^{2+}$  pump [65]. There are clear similarities between the physiological properties of the digestive vacuole and those of the acidic vacuole of plant cells. The question of the actual value of the pH in the digestive vacuole of the parasite remains a vexed one, and the various groups struggling with this have yet to reach any consensus.

### $\text{Ca}^{2+}$ stores and signals

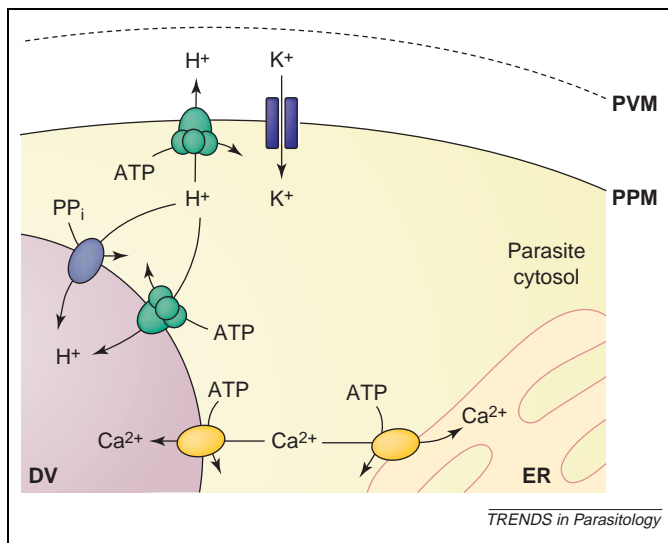
$\text{Ca}^{2+}$  plays a key role in intracellular signalling in eukaryotic cells.  $\text{Ca}^{2+}$  is known to be essential at several stages in the parasite lifecycle, but its roles, and its regulation within the parasite, are not well understood. In the hours immediately following invasion of the erythrocyte, there is a significant  $\text{Ca}^{2+}$  concentration maintained



**Figure 2.** Different models for the new permeability pathways induced by the parasite in the membrane of its host erythrocyte. The effects of a range of transport inhibitors on the transport of a range of different classes of solutes (including anions, neutral solutes and cations) were interpreted in terms of (a) a single anion-selective channel [39]; the results of at least some electrophysiological studies [41,42,50] have been largely consistent with this view. A recent analysis of the transport data, based on the assumption that the channels behaved as size-selective cylindrical pores, has led to the proposal (b) that there are two classes of channel – one permeable to monovalent anions and perhaps nucleosides, the other permeable to a broader range of solutes [47]. It has recently been reported that erythrocytes from cystic fibrosis patients lack a parasite-induced anion conductance, but show the same enhanced permeability to at least some organic solutes as parasitized erythrocytes from normal donors [51] and this might be interpreted as being consistent with this view. However, this finding has been disputed [50]. Lang and colleagues have described three discrete ion conductances active in parasitized erythrocytes (c) and have attributed these to: (i) an outwardly rectifying anion channel (ORAC) permeable to  $\text{Cl}^-$  and a range of organic solutes; (ii) an inwardly rectifying anion channel (IRAC) permeable to  $\text{Cl}^-$  and other inorganic anions; and (iii) a non-selective cation channel (NSCC) permeable to  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  [43–45]. Observation of ORAC currents in parasitized erythrocytes has been shown to be sensitive to the presence of serum in the medium [46].

within the parasitophorous vacuole, perhaps maintained by  $\text{Ca}^{2+}$  pumps originating from the erythrocyte membrane [66]. The parasite itself is known to have several distinct internal  $\text{Ca}^{2+}$  stores, including its acidic digestive vacuole [65] and endoplasmic reticulum (Figure 3), but the relationship between these stores, and their physiological roles, remain unclear. Several putative  $\text{Ca}^{2+}$  pumps have been identified in the parasite genome and one of these, PfATP6, is an orthologue of the sarco/endoplasmic reticulum  $\text{Ca}^{2+}$  ATPases. PfATP6 expressed in *Xenopus* oocytes is inhibited with high potency by artemisinins [67]. It has been proposed that it is this inhibition that underlies the antimalarial action of these compounds [67], although it has yet to be shown that the artemisinins actually release  $\text{Ca}^{2+}$  from the intracellular stores of the parasite *in situ*, as is the case for at least one other PfATP6 inhibitor, cyclopiiazonic acid [68].

Until very recently, all of the studies of intracellular  $\text{Ca}^{2+}$  regulation in the parasite entailed the use of chemical  $\text{Ca}^{2+}$  indicators. However, Billker *et al.* [69] have reported the successful generation of a *P. berghei* strain, expressing the  $\text{Ca}^{2+}$  indicator aequorin, together with GFP. The transgene products allow the sensitive detection of cytosolic  $\text{Ca}^{2+}$ , and it was shown that addition of xanthurenic acid (a molecule present in the mosquito midgut and known to induce differentiation of *Plasmodium* gametocytes) triggers an increase in cytosolic  $\text{Ca}^{2+}$ . Expression of protein-based ion indicators in plants has proven extremely useful in studies of plant cell physiology, and the demonstration that the same approach is feasible in *Plasmodium* is an important development.



**Figure 3.** Inorganic ion homeostasis in the intracellular parasite. The figure includes only those pathways for which there is direct physiological evidence. A V-type  $\text{H}^+$  ATPase in the parasite plasma membrane (PPM) extrudes  $\text{H}^+$  from the parasite [52], thereby generating a significant inward  $[\text{H}^+]$  gradient [53] and membrane potential [54]. A similar pump in the membrane of the digestive vacuole (DV) operates in parallel with a  $\text{H}^+$  pyrophosphatase, driving  $\text{H}^+$  into (and thereby acidifying) the vacuole [64]. A  $\text{Ba}^{2+}$ - and  $\text{Cs}^+$ -sensitive  $\text{K}^+$  channel in the parasite plasma membrane mediates the uptake of  $\text{K}^+$  [54]. The DV [65] and endoplasmic reticulum (ER) [67] take up  $\text{Ca}^{2+}$  through a  $\text{Ca}^{2+}$  ATPase. The number, nature and roles of the  $\text{Ca}^{2+}$  stores in the parasite are unclear. Similarly, the mechanism by which the parasite maintains a low cytosolic  $[\text{Na}^+]$  remains to be established. The PVM denotes the parasitophorous vacuole in which the intraerythrocytic parasite is enclosed.

## Transporters in drug resistance

As well as their roles in nutrient uptake, waste disposal and ion homeostasis, transporters are known to play a key role in the phenomenon of drug resistance in a wide range of organisms. In the malaria parasite, two putative transporter proteins have been implicated in the phenomenon of chloroquine resistance (CQR): the chloroquine resistance transporter (CRT) and the P-glycoprotein homologue 1 (Pgh1). CQ exerts its antimalarial effect by interfering with the mechanism by which potentially toxic haem monomers (originating from the degradation of haemoglobin) are detoxified by conversion to the crystalline and inert haemozoin in the digestive vacuole. It is thought that CQR is achieved through the reduction in the accumulation of CQ within the digestive vacuole. Both CRT and Pgh1 are on the digestive vacuole membrane. Mutations in CRT confer CQR on otherwise sensitive strains, whereas mutations in Pgh1 enhance resistance in parasites that have established CQR through an alternative mechanism (e.g. mutations in CRT). CRT is a member of the drug/metabolite transporter superfamily [70,71], whereas Pgh1 is a member of the ATP-binding cassette superfamily. Both are therefore related to known transporters; however, as yet, there has not been any direct demonstration of the transport function of either protein. Attempts to express and characterize both proteins in heterologous systems (e.g. yeast, *Xenopus* oocytes) are under way (e.g. Ref. [72,73]). It is likely that CQR-conferring mutations in the gene encoding CRT confer or enhance the ability of this protein to export CQ from the digestive vacuole, away from its site of action [71]. How mutations in the gene encoding Pgh1 might influence CQR is less clear.

Pgh1 is also implicated in the resistance of *P. falciparum* to mefloquine, with amplification of the gene encoding Pgh1 being associated with mefloquine resistance both *in vitro* and *in vivo* [74]. This has all the hallmarks of a classical upregulation of a drug efflux pump, as occurs in human cancer cells in which drug resistance is conferred by overexpression of the Pgh1 orthologue P-glycoprotein. Again, however, the transport of mefloquine by Pgh1 has yet to be demonstrated directly.

The physiological substrates and roles of CRT and Pgh1 in the parasite remain unclear. Whether the function of these proteins, and therefore the fitness of the parasite, is compromised by resistance-associated mutations or by alterations in expression level is presently under investigation by several groups. There is evidence from the field that CQ-resistant parasite strains are outgrown by CQ-sensitive forms when CQ usage in the community is reduced (e.g. Ref. [75]), and Hayward *et al.* have reported that replacement of CQR-enhancing mutations in Pgh1 with wild-type polymorphisms increases the rate of parasite growth *in vitro* [76]. Both observations are consistent with antimalarial drug resistance having some cost in terms of parasite fitness.

## Conclusion

With the emergence and spread of malaria parasites that are resistant to most of the antimalarial drugs presently available, there is an urgent need for the identification of

new drug targets and the development of novel anti-malarial strategies. The knowledge gained over recent years about parasite-specific organelles, metabolic pathways and membrane transport mechanisms will certainly facilitate future drug development. The detailed structural and functional analysis of parasite enzymes, and their comparison with their isofunctional counterparts in the host, paves the way for rational drug design. Exploiting knowledge gained about the transport processes operating in the infected cell might well enhance the success of these approaches. Directing a drug specifically to the infected cells, and to the parasites within, will enhance drug efficiency and decrease side-effects, and an understanding of the mechanisms underlying antimalarial drug resistance should help us to circumvent the emergence of resistance to new generations of antimalarials.

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### References

- Ralph, S.A. *et al.* (2004) Metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat. Rev. Microbiol.* 2, 203–216
- Foth, B.J. *et al.* (2003) Dissecting apicoplast targeting in the malaria parasite *Plasmodium falciparum*. *Science* 299, 705–708
- Gornicki, P. (2003) Apicoplast fatty acid biosynthesis as a target for medical intervention in apicomplexan parasites. *Int. J. Parasitol.* 33, 885–896
- Thomsen-Zieger, N. *et al.* (2003) Apicomplexan parasites contain a single lipoic acid synthase located in the plastid. *FEBS Lett.* 547, 80–86
- Dhanasekaran, S. *et al.* (2004) Delta-aminolevulinic acid dehydratase from *Plasmodium falciparum*: indigenous versus imported. *J. Biol. Chem.* 279, 6934–6942
- Seeber, F. (2003) Biosynthetic pathways of plastid-derived organelles as potential drug targets against parasitic apicomplexa. *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 3, 99–109
- Foth, B.J. and McFadden, G.I. (2003) The apicoplast: a plastid in *Plasmodium falciparum* and other Apicomplexan parasites. *Int. Rev. Cytol.* 224, 57–110
- Bender, A. *et al.* (2003) Properties and prediction of mitochondrial transit peptides from *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 132, 59–66
- Uyemura, S.A. *et al.* (2004) Oxidative phosphorylation and rotenone-insensitive malate- and NADH-quinone oxidoreductases in *Plasmodium yoelii yoelii* mitochondria *in situ*. *J. Biol. Chem.* 279, 385–393
- Wrenger, C. and Muller, S. (2003) Isocitrate dehydrogenase of *Plasmodium falciparum*. *Eur. J. Biochem.* 270, 1775–1783
- Ulschmid, K. *et al.* (2004) Adenylate kinase and GTP:AMP phosphotransferase of the malarial parasite *Plasmodium falciparum*. Central players in energy metabolism. *Mol. Biochem. Parasitol.* 136, 211–220
- Mi-Ichi, F. *et al.* (2003) Unique properties of respiratory chain in *Plasmodium falciparum* mitochondria. *Adv. Exp. Med. Biol.* 531, 117–133
- Vaidya, A.B. (2004) Mitochondrial and plastid functions as antimalarial drug targets. *Curr. Drug Targets Infect. Disord.* 4, 11–23
- Tarnchompoo, B. *et al.* (2002) Development of 2,4-diaminopyrimidines as antimalarials based on inhibition of the S108N and C59R+S108N mutants of dihydrofolate reductase from pyrimethamine-resistant *Plasmodium falciparum*. *J. Med. Chem.* 45, 1244–1252
- Sardarian, A. *et al.* (2003) Pyrimethamine analogs as strong inhibitors of double and quadruple mutants of dihydrofolate reductase in human malaria parasites. *Org. Biomol. Chem.* 1, 960–964
- Rastelli, G. *et al.* (2003) Structure of *Plasmodium vivax* dihydrofolate reductase determined by homology modeling and molecular dynamics refinement. *Bioorg. Med. Chem. Lett.* 13, 3257–3260
- Birkholtz, L.M. *et al.* (2004) Parasite-specific inserts in the bifunctional *S*-adenosylmethionine decarboxylase/ornithine decarboxylase of *Plasmodium falciparum* modulate catalytic activities and domain interactions. *Biochem. J.* 377, 439–448
- Shi, W. *et al.* (2004) *Plasmodium falciparum* purine nucleoside phosphorylase: crystal structures, immucillin inhibitors, and dual catalytic function. *J. Biol. Chem.* 279, 18103–18106
- Kaiser, A. *et al.* (2003) Targeting enzymes involved in spermidine metabolism of parasitic protozoa – a possible new strategy for anti-parasitic treatment. *Parasitol. Res.* 91, 508–516
- Berglez, J. *et al.* (2004) Analysis in *Escherichia coli* of *Plasmodium falciparum* dihydropteroate synthase (DHPS) alleles implicated in resistance to sulfadoxine. *Int. J. Parasitol.* 34, 95–100
- Kasekarn, W. *et al.* (2003) Molecular characterization of bifunctional hydroxymethyl-dihydropterin pyrophosphokinase-dihydropteriate synthase from *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 137, 43–53
- Wang, P. *et al.* (2002) Rapid positive selection of stable integrants following transfection of *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 123, 1–10
- Wang, P. *et al.* (2004) Transfection studies to explore essential folate metabolism and antifolate drug synergy in the human malaria parasite *Plasmodium falciparum*. *Mol. Microbiol.* 51, 1425–1438
- Saliba, K.J. and Kirk, K. (2001) H<sup>+</sup>-coupled pantothenate transport in the intracellular malaria parasite. *J. Biol. Chem.* 276, 18115–18121
- Saliba, K.J. *et al.* (2003) Pantothenate utilization as a target for antimalarial chemotherapy. *Exp. Parasitol.* 105, 22
- Rahlfs, S. *et al.* (2002) The thioredoxin system of *Plasmodium falciparum* and other parasites. *Cell. Mol. Life Sci.* 59, 1024–1041
- Becker, K. *et al.* (2003) Glutathione – functions and metabolism in the malarial parasite *Plasmodium falciparum*. *Biol. Chem.* 384, 551–566
- Becker, K. *et al.* (2004) Oxidative stress in malaria parasite-infected erythrocytes: host–parasite interactions. *Int. J. Parasitol.* 34, 163–189
- Becker, K. *et al.* (2003) Plasmoredoxin, a novel redox-active protein unique for malarial parasites. *Eur. J. Biochem.* 270, 1057–1064
- Fritz-Wolf, K. *et al.* (2003) X-ray structure of glutathione *S*-transferase from the malarial parasite *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13821–13826
- Sarma, G.N. *et al.* (2003) Glutathione reductase of the malarial parasite *Plasmodium falciparum*: crystal structure and inhibitor development. *J. Mol. Biol.* 328, 893–907
- Schirmer, R.H. *et al.* (2003) Methylene blue as an antimalarial agent. *Redox Rep.* 8, 272–275
- Iozef, R. *et al.* (2003) Glyoxalase I of the malarial parasite *Plasmodium falciparum*: evidence for subunit fusion. *FEBS Lett.* 554, 284–288
- Bustamante, L.Y. *et al.* (2004) Dual-function stem molecular beacons to assess mRNA expression in AT-rich transcripts of *Plasmodium falciparum*. *Biotechniques* 36, 488–492
- Campanale, N. *et al.* (2003) Identification and characterization of heme-interacting proteins in the malaria parasite, *Plasmodium falciparum*. *J. Biol. Chem.* 278, 27354–27361
- Cameron, A. *et al.* (2004) Identification and activity of a series of azole-based compounds with lactate dehydrogenase-directed anti-malarial activity. *J. Biol. Chem.* 279, 31429–31439
- Saliba, K.J. *et al.* (1998) Transport and metabolism of the essential vitamin pantothenic acid in human erythrocytes infected with the malaria parasite *Plasmodium falciparum*. *J. Biol. Chem.* 273, 10190–10195
- Ginsburg, H. *et al.* (1985) Characterization of permeation pathways appearing in the host membrane of *Plasmodium falciparum* infected red blood cells. *Mol. Biochem. Parasitol.* 14, 313–322
- Kirk, K. *et al.* (1994) Transport of diverse substrates into malaria-infected erythrocytes via a pathway showing functional characteristics of a chloride channel. *J. Biol. Chem.* 269, 3339–3347
- Staines, H.M. *et al.* (2004) Furosemide analogues as potent inhibitors of the new permeability pathways of *Plasmodium falciparum*-infected human erythrocytes. *Mol. Biochem. Parasitol.* 133, 315–318
- Desai, S.A. *et al.* (2000) A voltage-dependent channel involved in nutrient uptake by red blood cells infected with the malaria parasite. *Nature* 406, 1001–1005

- 42 Egée, S. *et al.* (2002) A stretch-activated anion channel is up-regulated by the malaria parasite *Plasmodium falciparum*. *J. Physiol.* 542, 795–801
- 43 Huber, S.M. *et al.* (2002) *Plasmodium falciparum* activates endogenous Cl<sup>-</sup> channels of human erythrocytes by membrane oxidation. *EMBO J.* 21, 22–30
- 44 Duranton, C. *et al.* (2003) Electrophysiological properties of the *Plasmodium falciparum*-induced cation conductance of human erythrocytes. *Cell. Physiol. Biochem.* 13, 189–198
- 45 Duranton, C. *et al.* (2004) Organic osmolyte permeabilities of the malaria-induced anion conductances in human erythrocytes. *J. Gen. Physiol.* 123, 417–426
- 46 Staines, H.M. *et al.* (2003) Modulation of whole-cell currents in *Plasmodium falciparum*-infected human red blood cells by holding potential and serum. *J. Physiol.* 552, 177–183
- 47 Ginsburg, H. and Stein, W.D. (2004) The new permeability pathways induced by the malaria parasite in the membrane of the infected erythrocyte: comparison of results using different experimental techniques. *J. Membr. Biol.* 197, 113–134
- 48 Thomas, S.L. and Lew, V.L. (2004) *Plasmodium falciparum* and the permeation pathway of the host red blood cell. *Trends Parasitol.* 20, 122–125
- 49 Staines, H.M. *et al.* (2004) *Plasmodium falciparum*-induced channels. *Int. J. Parasitol.* 34, 665–673
- 50 Alkhalil, A. *et al.* *Plasmodium falciparum* likely encodes the principal anion channel on infected human erythrocytes. *Blood* (in press)
- 51 Verloo, P. *et al.* (2004) *Plasmodium falciparum*-activated chloride channels are defective in erythrocytes from cystic fibrosis patients. *J. Biol. Chem.* 279, 10316–10322
- 52 Saliba, K.J. and Kirk, K. (1999) pH regulation in the intracellular malaria parasite, *Plasmodium falciparum*: H<sup>+</sup> extrusion via a V-type H<sup>+</sup>-ATPase. *J. Biol. Chem.* 274, 33213–33219
- 53 Hayashi, M. *et al.* (2000) Vacuolar H<sup>+</sup>-ATPase localized in plasma membranes of malaria parasite cells, *Plasmodium falciparum*, is involved in regional acidification of parasitised erythrocytes. *J. Biol. Chem.* 275, 34353–34358
- 54 Allen, R.J. and Kirk, K. (2004) The membrane potential of the intraerythrocytic malaria parasite *Plasmodium falciparum*. *J. Biol. Chem.* 279, 11264–11272
- 55 Krishna, S. *et al.* (2002) Transport processes in *Plasmodium falciparum*-infected erythrocytes: potential as new drug targets. *Int. J. Parasitol.* 32, 1567–1573
- 56 Biagini, G.A. *et al.* Characterization of the choline carrier of *Plasmodium falciparum*: a route for the selective delivery of novel antimalarial drugs. *Blood* (in press)
- 57 Lehane, A.M. *et al.* (2004) Choline uptake into the malaria parasite is energized by the membrane potential. *Biochem. Biophys. Res. Commun.* 320, 311–317
- 58 Wengelnik, K. *et al.* (2002) A class of potent antimalarials and their specific accumulation in infected erythrocytes. *Science* 295, 1311–1314
- 59 Ellekvist, P. *et al.* (2004) Molecular cloning of a K<sup>+</sup> channel from the malaria parasite *Plasmodium falciparum*. *Biochem. Biophys. Res. Commun.* 318, 477–484
- 60 Joet, T. *et al.* (2003) Validation of the hexose transporter of *Plasmodium falciparum* as a novel drug target. *Proc. Natl. Acad. Sci. U. S. A.* 100, 7476–7479
- 61 Saliba, K.J. *et al.* (2004) Inhibition of hexose transport and abrogation of pH homeostasis in the intraerythrocytic malaria parasite by an O-3-hexose derivative. *FEBS Lett.* 570, 93–96
- 62 Gardner, M.J. *et al.* (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 419, 498–511
- 63 Martin, R.E. *et al.* (2003) Parasite permeomics: an overview of membrane transport proteins in *Plasmodium falciparum*. *Exp. Parasitol.* 105, 56
- 64 Saliba, K.J. *et al.* (2003) Acidification of the malaria parasite's digestive vacuole by a H<sup>+</sup>-ATPase and a H<sup>+</sup>-pyrophosphatase. *J. Biol. Chem.* 278, 5605–5612
- 65 Biagini, G.A. *et al.* (2003) The digestive food vacuole of the malaria parasite is a dynamic intracellular Ca<sup>2+</sup> store. *J. Biol. Chem.* 278, 27910–27915
- 66 Gazarini, M.L. *et al.* (2003) Calcium signaling in a low calcium environment: how the intracellular malaria parasite solves the problem. *J. Cell Biol.* 161, 103–110
- 67 Eckstein-Ludwig, U. *et al.* (2003) Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature* 424, 957–961
- 68 Alleva, L.M. and Kirk, K. (2001) Calcium regulation in the intraerythrocytic malaria parasite *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 117, 121–128
- 69 Billker, O. *et al.* (2004) Calcium and a calcium-dependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. *Cell* 117, 503–514
- 70 Tran, C.V. and Saier, M.H., Jr. (2004) The principal chloroquine resistance protein of *Plasmodium falciparum* is a member of the drug/metabolite transporter superfamily. *Microbiology* 150, 1–3
- 71 Martin, R.E. and Kirk, K. (2004) The malaria parasite's chloroquine resistance transporter is a member of the drug/metabolite transporter superfamily. *Mol. Biol. Evol.* 21, 1938–1949
- 72 Zhang, H. *et al.* (2002) Analysis of the antimalarial drug resistance protein Pfert expressed in yeast. *J. Biol. Chem.* 277, 49767–49775
- 73 Nessler, S. *et al.* (2004) Evidence for activation of endogenous transporters in *Xenopus laevis* oocytes expressing the *Plasmodium falciparum* chloroquine resistance transporter, PfCRT. *J. Biol. Chem.* 279, 39438–39446
- 74 Price, R.N. *et al.* (2004) Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 copy number. *Lancet* 364, 438–447
- 75 Abdel-Muhsin, A.M. *et al.* (2004) Evolution of drug-resistance genes in *Plasmodium falciparum* in an area of seasonal malaria transmission in Eastern Sudan. *J. Infect. Dis.* 189, 1239–1244
- 76 Hayward, R. *et al.* (2003) Mutations in Pgh1 associated with chloroquine resistance reduce the fitness of *Plasmodium falciparum*. *Exp. Parasitol.* 105, 44

### Food for thought: how improved nutrition could help to reduce the malaria burden

A review of recent data from malaria-endemic regions has suggested that improved child nutrition could prevent more malaria-related illnesses and deaths than previously thought.

Researchers from the Johns Hopkins Bloomberg School of Public Health (<http://www.jhsph.edu/>) found that mildly malnourished children were twice as likely to die from malaria when compared with children who are not undernourished, whereas moderately malnourished children were four times more likely to die. Severely malnourished children were nine times more likely to die.

Zinc and vitamin A deficiencies are commonplace in malaria-endemic regions and a lack of these key nutrients compromises a child's ability to fight infection. Zinc improves growth and enhances the body's ability to respond to infection, and vitamin A plays an essential role in the immune response and is believed to be necessary for host resistance to malaria.

According to global burden of disease data published in early 2004, nearly 550 000 malaria-related deaths each year are attributable to underweight children under the age of five years. Addressing and improving the diets of children aged under five years could better equip them to fight and survive malaria infection.

For more information, please go to:  
[http://www.jhsph.edu/Press\\_Room/Press\\_Releases/PR\\_2004/Caulfield\\_malaria.html](http://www.jhsph.edu/Press_Room/Press_Releases/PR_2004/Caulfield_malaria.html)

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