Role of phospholipid synthesis in the development and differentiation of malaria parasites in the blood

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The life cycle of malaria parasites in both their mammalian host and mosquito vector consists of multiple developmental stages that ensure proper replication and progeny survival. The transition between these stages is fueled by nutrients scavenged from the host and fed into specialized metabolic pathways of the parasite. One such pathway is used by Plasmodium falciparum, which causes the most severe form of human malaria, to synthesize its major phospholipids, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. Much is known about the enzymes involved in the synthesis of these phospholipids, and recent advances in genetic engineering, single-cell RNA-Seq analyses, and drug screening have provided new perspectives on the importance of some of these enzymes in parasite development and sexual differentiation and have identified targets for the development of new antimalarial drugs. This Minireview focuses on two phospholipid biosynthesis enzymes of P. falciparum that catalyze phosphoethanolamine transmethylation (PfPMT) and phosphatidylserine decarboxylation (PfPSD) during the blood stages of the parasite. We also discuss our current understanding of the biochemical, structural, and biological functions of these enzymes and highlight efforts to use them as antimalarial drug targets.

Malaria and the challenging search for effective antimalarial chemotherapy

Malaria is a mosquito-borne parasitic disease caused by protozoan parasites of the genus Plasmodium and is one of the leading causes of death throughout human history. The disease is endemic in 91 countries with the World Health Organization African Region carrying the biggest burden of morbidity and mortality (1). Of the Plasmodium species that infect humans, Plasmodium falciparum and Plasmodium vivax account for the overall majority of malaria clinical cases, hospital stays, and death (1, 2). In 2016, these parasites were responsible for ∼216 million clinical cases and ∼445,000 deaths (1). Thanks to major international efforts aimed at implementing improved policies for control of mosquito populations, the wide use of bed nets, and the application of new therapeutic strategies, this mortality rate represents a drop of more than 50% from the ∼839,000 deaths recorded in 2000 (1, 3). However, despite this success, the death toll is still unacceptably high. Emerging drug resistance to first line therapies and the high cost of drugs continue to add more health and economic burden on the affected populations (1, 4, 5). The development of an effective vaccine continues to be both scientifically and technically challenging. Among multiple candidates currently in development, RTS,S/AS01 (or MosquirixTM), a recombinant protein-based vaccine that targets the major circumsporozoite protein of P. falciparum, has shown the most promise so far (6). In the absence of a vaccine with high effectiveness among the overall population, there continues to be a need for new and affordable therapies and novel therapeutic strategies to treat the disease.

Plasmodium parasites have a complex life cycle in the mosquito vector and humans, involving multiple developmental stages, different morphological, biochemical, and metabolic requirements, and well-controlled and highly coordinated gene expression and regulatory mechanisms (7, 8). Following injection of the parasite into the skin of the human host by an infected female Anopheles mosquito, the parasite undergoes rapid multiplication within liver hepatocytes to produce thousands of merozoites, which are packed into host cell membrane-derived vesicles (merosomes) and safely transported past the resident macrophages (Kupffer cells) into the liver sinusoids where their invasion of the erythrocytes begins (9–12).

Within the erythrocytes, each merozoite grows to several times its original size before dividing asexually via schizogony to produce 16–32 new blood merozoites (13, 14). The intracellular erythrocyte life cycle ends with the rupture of the host cell. The repeated cycles of invasion and destruction of host erythrocytes are directly linked to the pathology and symptoms of the disease, which include fever, chills, and fatigue (15, 16).

The orchestration of the intraerythrocytic schizogony requires a complete parasitic reorganization of the metabolically reduced and terminally differentiated host erythrocyte to ensure protection against immune attacks and to facilitate nutrient supply to fuel parasite development and replication (1, 17–20). While long recognized as critical structural components for parasite development and attractive therapeutic targets, phospholipids and their by-products have also emerged (21) as major signaling molecules that control development and

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Table 1
Phospholipid composition of different eukaryotic cells

| Organism/cell | PC (%) | PE (%) | PS (%) | Refs. |
|---------------|--------|--------|--------|-------|
| Uninfected erythrocytes | 30–40 | 25–35 | 10–20% | 33 |
| P. falciparum-infected erythrocytes and free parasites | 20–55 | 15–40 | 4–15% | 25, 33, 37 |
| Tryptococcus brucei | 45–60 | 10–20 | <4% | 36 |
| Toxoplasma gondii (free parasites) | 75% | 10% | 6% | 34 |
| Aspergillus niger | 51% | 28.5% | 5% | 35 |
| Candida albicans | 40% | 25.3% | 12% | 35 |
| Cryptococcus neoformans | 49% | 28% | 8% | 35 |
| Microsporum gypseum | 23.1% | 29.8% | 19.4% | 35 |

The comparison of lipid composition of different eukaryotic cells and uninfected erythrocytes is shown.

Differentiation processes during Plasmodium intraerythrocytic cycle (21–26). The rapid generation of abundant parasitic progeny requires the appropriate amount of suitable lipid species at the proper compartment and at the right time to establish an active membrane biogenesis, which leads to a dramatically elevated lipid metabolism during the intraerythrocytic schizogony (22, 27). To acquire the necessary lipid species for different compartments, the parasite either synthesizes them de novo from previously produced metabolites or uses exogenous sources such as the erythrocyte membrane or the human plasma. This results in a 6-fold increase in the relative levels of phospholipids in the infected erythrocyte (24, 27, 28). The dependence on phospholipids for rapid parasite multiplication and the uniqueness of some of the steps in the pathways of Plasmodium lipid metabolism create significant opportunities for the identification of antimalarial drug targets (22, 24, 27).

Structural, developmental, and signaling functions of phospholipid biosynthesis in P. falciparum

Asexual blood stages and gametocytes of Plasmodium parasites are able to scavenge or synthesize up to 300 different lipid species to facilitate growth, proliferation, transmission, and sexual reproduction (24). The phospholipid classes, phosphatidylcholine (PC),3 phosphatidylethanolamine (PE), and phosphatidylserine (PS), are the major lipid components that define Plasmodium membranes (21–23, 25, 29–32). In the uninfected erythrocyte, PC, PE, and PS constitute 30–40, 25–35, and 10–20% of the total phospholipids, respectively, whereas in P. falciparum-infected erythrocytes and in purified parasites, these major phospholipids constitute 20–55, 15–40, and 4–15% of the total phospholipids, respectively (Table 1) (25, 33).

The reported higher levels of PE in P. falciparum membranes compared with the membranes of other eukaryotes (Table 1) have been proposed to be largely due to the inability of the parasite to directly convert PE to PC (22, 23). However, P. falciparum is able to generate phosphocholine, a precursor for the synthesis of PC, from phosphoethanolamine via the PMT pathway (see below). With the exception of a few pathways, which have been identified through metabolic and genetic analyses, most components of the PC, PE, and PS biosynthetic machineries have been identified by searching for homologs in the Plasmodium genome databases of well-characterized enzymes from yeast, plants, and other eukaryotes (22, 30). These pathways are outlined in Fig. 1.

Phosphatidylcholine

In most eukaryotic membranes (see Table 1), PC accounts for more than 50% of phospholipids and spontaneously self-organizes to a planar bilayer (38, 39). In addition to its structural role, PC can also modulate cellular signaling functions because its hydrolysis by phospholipases leads to the formation of the second messenger diacylglycerol (DAG), which is critical for activation of specific classes of protein kinases (40, 41). Changes in cellular PC levels have been shown to alter cell proliferation, differentiation, as well as membrane movement (42). Studies in malaria parasites have shown that PC is the major phospholipid in Plasmodium membranes during both liver stage and intra-erythrocytic schizogony (22, 30, 43, 44).

One of the two routes used to synthesize PC in Plasmodium parasites is the cytidine diphosphate- or CDP-choline branch of the Kennedy pathway (25, 28, 33, 38, 45). Metabolomic analyses showed that 89% of PC is generated by the parasite from choline via this particular pathway (33). To enter the Kennedy pathway, choline is first transported into the parasite by an as yet unidentified transporter and then phosphorylated by choline kinase to form phosphocholine, which is subsequently used as a precursor to form CDP-choline and finally PC, by the 1,2-diacylglycerol cholinephosphotransferase (45). PC can also be synthesized by the PMT pathway, which uses three sequential methylations of phosphoethanolamine (via S-adenosylmethionine donors) to form phosphocholine, catalyzed by the parasite phosphoethanolamine methyltransferase (PfPMT) (22, 46–48). Metabolomic analyses indicate that 11% of total PC in P. falciparum is generated from the PMT pathway (33). Analysis of available genome databases showed that among protozoan parasites, PfPMT homologs are found primarily among Plasmodium species that infect humans (P. vivax and P. knowlesi), primates (P. knowlesi and Plasmodium reichenowi), and birds (P. gallinaceum) (49–51). Orthologs of PfPMT have also been found in proteobacteria (Burkholderia pseudomallei and Burkholderia oklahomensis), many species of plants, two species of African clawed frogs ( Xenopus laevis and Xenopus tropicalis), nematodes (Caenorhabditis elegans and Caenorhabditis briggsae), zebrafish ( Danio rerio), the Florida lancelet ( Brachistoma floridae), and the protozoan fungus Phytophthora infestans (49). Interestingly, no PMT orthologs

3 The abbreviations used are: PC, phosphatidylcholine; PE, phosphatidylethanolamine; CDP, cytidine diphosphate; DAG, diacylglycerol; lyso-PC, lyso-phosphatidylcholine; PSD, PS decarboxylase; PNSM, lyso-PC-dependent phospholipase C (SM/PLC-phospholipase C or PLC); PPSD, P. falciparum phosphatidylethanolamine synthetase; PIPMT, P. falciparum phosphoethanolamine methyltransferase; PS, phosphatidylserine; PIPMT, P. vivax phosphoethanolamine methyltransferase; PMT, phosphoethanolamine methyltransferase; TM, transmembrane domain; 7CPOA, 7-chloro-N-(4-ethoxyphenyl)-4-quinolinamine.
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Figure 1. Lipid synthetic pathways in the asexual stages of Plasmodium. The following abbreviations are used: RBCM, red blood cell membrane; RBCC, red blood cell cytosol; PPM, parasite plasma membrane; DV, digestive vacuole; HZ, hemozoin; PAA, peptides and amino acids; PPS, P. falciparum PS synthase; PPSD, P. falciparum serine decarboxylase; PFECT, P. falciparum choline transporter; PDPLC, P. falciparum phospholipase C; PPSD, P. falciparum PS decarboxylase; PICT, P. falciparum choline phosphotransferase; PPD-LC, lyso-PC–dependent phospholipase C; PNSM, P. falciparum neutral sphingomyelinase; LD-PLD, lyso-PC–dependent phospholipase D. Dashed arrows represent putative routes of transport and metabolism. The “?” indicates pathways and enzymes that might exist or catalyze certain reactions respectively but that have not been identified yet.

are found in mammals, making them attractive targets for selective therapies (49). The 3D structure of PfPMT and subsequently that of PvPMT (P. vivax phosphoethanolamine methyltransferase) and PKPMT (P. knowlesi phosphoethanolamine methyltransferase) showed high conservation, functional activity, and inhibition by the same drugs (29, 52, 53). Like other methyltransferases known to be inhibited by the 4-aminoquinoline, amodiaquine, PfPMT was also inhibited by this compound (Table 2) (54). The specificity of interaction of amodiaquine with the enzyme was further demonstrated using NMR spectroscopy (54). The compound was found to induce major conformational changes, whereas its analog chloroquine had no effect on the enzyme (54). Structural analyses of PvPMT with amodiaquine further showed that the drug is an allosteric inhibitor targeting a cleft located distal to the active site (43).

P. falciparum ppmTΔ parasites lacking the PpmT gene were found to be viable even in the absence of exogenous choline, although their growth was severely reduced (Table 2) (23). TLC analyses showed that these parasites produce PC in the absence of choline, suggesting that an alternative source of choline or a choline-containing precursor is also available to the parasite (23). Accordingly, a recent study by Brancucci et al. (55) showed that P. falciparum actively transports lysophosphatidylethanolamine (lyso-PC) from host plasma to generate PC (Fig. 1). Although the mechanism of uptake and mode of utilization of lyso-PC by the parasite remain to be determined, it is possible that lyso-PC is either hydrolyzed to form a precursor that then enters the CDP-choline pathway to form PC or is directly acylated to form PC (Fig. 1). In yeast, lyso-PC is transported by the phospholipid flippases, Dnf1p and Dnf2p, which are P4 ATPases (56, 57). A second protein, Lem3p, also plays a role in the transport of lyso-PC as a noncatalytic subunit of Dnf1p–Lem3p and Dnf2p–Lemp3p complex. Because the crystal structure of the P4 ATPase is not yet available, the structural basis of lyso-PC uptake remains unknown. However, several models have been proposed based on extensive mutagenesis and computer simulation analyses using crystal structures of cation--transporting P-type ATPases (58–61). Among them, the two-gate model has been proposed for phospholipid translocation by Dnf1p (58). First, the phospholipid headgroups slide through the entry gate formed by the transmembrane domains (TMs) on the extracellular side and then reach the exit gate formed through the other TMs toward the cytosol. Once inside the cell, acylation of lyso-PC is catalyzed by Ale1p (acyltransferase for lysophosphatidylethanolamine) (57, 62). A BLAST search for homologs of these proteins in the P. falciparum genome database (http://plasmodb.org/plasmo/) identified several homologs of Lem3p and Dnf1p/2p, but no homologs of Ale1p could be found.

Phosphatidylethanolaminol

PE can be found in the membranes of all eukaryotic and prokaryotic cells and is a key factor in several processes such as membrane fusion or cytokinesis and can further serve as a donor of the ethanolamine moiety that covalently modifies various proteins (63, 64). PE also increases the membrane curvature, which is crucial for membrane budding, as well as fusion and fission processes (29, 65). PE metabolism has been implicated in important cellular processes such as autophagy, signal- and viral replication (66–70) and in human diseases such as Alzheimer's disease, Parkinson's disease, and nonalcoholic liver disease (71). The biosynthesis of PE in Plasmodium parasites

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can occur either from ethanolamine via the cytidine diphosphate- or CDP-ethanolamine (Kennedy) pathway or from PS via decarboxylation (Fig. 1) (29, 31, 33). The PE branch of the Kennedy pathway uses three sequential steps catalyzed by parasite enzymes to generate PE de novo from ethanolamine (72).

The ethanolamine kinase of the Kennedy pathway uses ATP to phosphorylate ethanolamine, whereas the phosphoethanolamine cytidylyltransferase uses CTP to generate the intermediate is then used in conjunction with DAG, by the 1,2-diacylglycerol ethanolaminephosphotransferase, to generate PE (45). PE synthesis from PS is usually found in the inner leaflet of the plasma membrane of healthy mammalian cells and plays a crucial role in apoptosis, erythrocyte senescence, blood coagulation, as well as targeting and function of various intracellular signaling proteins (64, 87–89). In malaria parasites, PS has been shown to play a role in P. falciparum-established erythrocyte cytoadhesion (32). Interestingly, PS is also particularly enriched in microvesicles that can be found in elevated levels within the blood of P. falciparum-infected patients (24). These microvesicles have been implicated in intercellular communication as well as gametocytogenesis (24). A recent study has further shown that the parasite actively hampers the presentation of PS on the outer membrane leaflet of the plasma membrane of liver cells during merozoite formation to avoid the phagocytic clearance of the parasitic progeny (90).

The synthesis of PS by Plasmodium parasites requires serine that is either directly imported from the host plasma or obtained from hemoglobin degradation in the digestive vacuole by parasite proteases (Fig. 1) (22, 23, 31, 33). PpPS (P. falciparum phosphatidylinerse synthase) then uses the scavenged serine to generate PS. PS further serves as a precursor for the synthesis of PE.

**Role of phospholipid synthesis in Plasmodium development and sexual differentiation**

The first evidence that phospholipids play more than just a housekeeping function in P. falciparum came from studies by Witola et al. (23) on the characterization of knockout parasites lacking the PfPMT gene. These mutant parasites had severe growth defects, even in the presence of high concentrations of choline (23). Furthermore, mature schizonts isolated from

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**Table 2**

**Overview of inhibitors and genetic knockouts of lipid synthetic enzymes**

The following abbreviations are used: PfECT, P. falciparum CTP:phosphoethanolamine cytidylyltransferase; PfEK, P. falciparum ethanolamine kinase; PfPMT, P. falciparum phosphatidylserine synthase; PfPSD, P. falciparum PS decarboxylase; PfPSS, P. falciparum phosphatidylserine synthase; PfPLC, P. falciparum choline/ethanolamine-phosphotransferase; PfPMT, P. falciparum phosphoethanolamine methyltransferase; LD-PLC, lyso-PC– dependent phospholipase C; PfNSM, lyso-PC– dependent phospholipase C (SM/LCPL-phospholipase choline transporter; PfCCT, proteolytic cleavage into a large (Asp–His–Ser) protease family (74) and undergoes auto-endo-

| Enzyme | Knockout lethality | Pharmacological inhibition | Putative compounds | Refs. |
|--------|-------------------|---------------------------|--------------------|------|
| PfEK   | NA                | +                         | 2-Amino-1–butanol, bis-thiazolium T3 | 78   |
| PfECT  | NA                | NA                        | NA                 |      |
| PfCT   | NA                | +                         | Amiodarone, bepridil, possibly pentamine, P16, and HC-3 | 86   |
| PICT   | NA                | +                         | Hemicholinium-3, bis-thiazolium T3 | 78–80 |
| PICK   | NA                | +                         | PG12               | 81   |
| PICCCT | +                 | +                         | T4                 | 81, 82 |
| PICCEPT| NA                | +                         | Amodiaquine, NSC-158011 | 21, 23, 54 |
| PIPMT  | NA                | +                         | NA                 |      |
| PIPSS  | NA                | +                         | NA                 |      |
| PIPLC  | NA                | +                         | NA                 |      |
| PIPSD  | NA                | +                         | 7CPQA analog (MMV007285) | 29, 85 |
| PIPSNM (LD-PLC) | NA       | +                         | Scyphostatin       | 84   |
| PIPSD  | NA                | NA                        | NA                 |      |

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Recent studies have shown that the enzyme belongs to the DHS improved its solubility while preserving catalytic activity (73). Biochemical characterization of PpPSD showed that the enzyme is less hydrophobic than other PSD enzymes and is recovered in both soluble and membrane-associated compartments when expressed in yeast (73). Deletion of the N-terminal membrane domain of the protein greatly improved its solubility while preserving catalytic activity (73). Recent studies have shown that the enzyme belongs to the DHS (Asp–His–Ser) protease family (74) and undergoes auto-endopeptolytic cleavage into a large β-subunit and a smaller α-subunit with a pyruvyl prosthetic group (73, 75, 76).

Metabolic studies in P. knowlesi suggested that besides ethanolamine uptake, a parasite-encoded serine decarboxylase might exist which could produce ethanolamine from serine (31). This reaction has previously been shown to occur exclusively in plants (77). However, no homologs of plant serine decarboxylases could be found in the P. falciparum genome, and attempts to isolate this enzyme from parasite extracts or to identify the malarial gene by functional complementation in yeast have not been successful (73). Biochemical assays further excluded the PpPSD enzyme as a possible decarboxylase of serine (29). One possible explanation of the available data is that Plasmodium lacks the ability for direct decarboxylation of serine and that the formation of ethanolamine from serine as shown by metabolic analyses in P. knowlesi is a result of PE turnover. In this case, serine is first incorporated into PS, and the resulting PS is then converted into PE, which is subsequently hydrolyzed to produce ethanolamine.

Genetic studies in P. falciparum and rodent parasites suggest that the PSD function might be essential for parasite development in the absence of exogenous ethanolamine (Table 2) (29). This has so far been supported by pharmacological studies with inhibitors that target this enzyme (Table 2) (29). However, further studies using gene editing technologies, conditional knockouts, or more specific inhibitors are needed to evaluate the importance of this step in parasite development and differentiation.

**Phosphatidylinerse**

PS is usually found in the inner leaflet of the lipid bilayer of the plasma membrane of healthy mammalian cells and plays a crucial role in apoptosis, erythrocyte senescence, blood coagulation, as well as targeting and function of various intracellular signaling proteins (64, 87–89). In malaria parasites, PS has been shown to play a role in P. falciparum-established erythrocyte cytoadhesion (32). Interestingly, PS is also particularly enriched in microvesicles that can be found in elevated levels within the blood of P. falciparum-infected patients (24). These microvesicles have been implicated in intercellular communication as well as gametocytogenesis (24). A recent study has further shown that the parasite actively hampers the presentation of PS on the outer membrane leaflet of the plasma membrane of liver cells during merozoite formation to avoid the phagocytic clearance of the parasitic progeny (90).

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these knockout parasites produced a smaller number of merozoites and displayed an altered pattern of nuclear division compared with WT parasites (23). Finally, these knockout parasites were severely altered in their commitment to sexual differentiation and could not be transmitted to mosquitoes (21). However, these defects could not be complemented by choline supplementation, implying that either pools of PC derived from the PMT pathway or intermediates of the methylation reactions may play a critical role in these processes.

Recent studies in P. falciparum have shown that lyso-PC is a major substrate of parasite phospholipid metabolism that further acts as an environmental sensor (55). By degradative processes, lyso-PC is also a major source of the Kennedy pathway components choline and fatty acids, which are needed to fuel the proper biosynthesis of PC (55). In case of lyso-PC limitation between 34 and 38 h after red blood cell invasion, the parasite responds with the production of less merozoites during the development of the blood stages (55, 91). Low lyso-PC conditions in the external medium further trigger the expression of the transcriptional regulator of irreversible sexual commitment and gametocytogenesis, Apz2G, whereas the expression of other key players such as PfPMT (especially in already committed cells) and other transcriptional factors are up-regulated (55, 92, 93). These recent findings suggest that lyso-PC controls the fate of P. falciparum by facilitating the repression of sexual differentiation. Interestingly, depletion of lyso-PC in the rodent parasite Plasmodium berghei, which lacks PMT function and other factors that are regulated by lyso-PC levels, had no effect on the sexual differentiation of the parasite (50, 55).

Summary and future directions

Important milestones have been achieved over the past several years in understanding lipid metabolism and its role in P. falciparum development and differentiation. The recent advances in metabolomic and genetic analyses will undoubtedly unravel more secrets about these processes and identify ideal targets for the development of new antimalarials.

The unique enzyme PfPMT has already been identified as an important key player in the lipid metabolism of P. falciparum parasites by Witola et al. (23). The knockout of PfPMT had major effects on growth and survival of the parasites, suggesting that PfPMT could be a promising target for antimalarial chemotherapy (23). Two studies showed that the antimalarial 4-aminoquinoline, amodiaquine, and four additional compounds (NSC-641296, NSC-668394, NSC-322341, and NSC-158011) were able to inhibit PfPMT activity (Table 2) (21, 54). NSC-158011 appeared to be a particularly promising candidate because it acts as a competitive inhibitor in the transmethylation reaction and inhibits PfPMT activity as well as PC biosynthesis in vivo (21).

Recent studies by Brancucci et al. (55) highlighted the important role of lyso-PC in P. falciparum sexual differentiation. However, the mechanism by which lyso-PC is transported from the host serum into the parasite remains to be investigated. The lyso-PC uptake might depend on the developmental stage of the parasite or even the age of the infected erythrocyte (14, 94). The parasite might also encounter different lyso-PC concentrations within various tissues while traveling with the host erythrocyte during its journey through the body as a juvenile ring or during sequestration as a mature trophozoite or a schizont (18, 95). Mature P. falciparum blood stages secrete and present cyttoadhesins on the surface of the infected erythrocytes, enabling these cells to become sequestered in the deep vascular bed of inner organs to avoid splenic clearance (18). Brancucci et al. (55) reported that parasite-induced sequestration in intra- and extravascular spaces might cause variation in lyso-PC exposure. They further observed dramatically reduced levels of lyso-PC in bone marrow fluids, where high densities of gametocytes can be found (96). However, gametocyte levels can also be high in the brain, gut, heart, and spleen, and it needs to be determined whether the localization of the sexual stages directly correlates with the lyso-PC levels within those organs (96).

Future studies are also needed to investigate why some of the asexual parasites appear to be unaffected when lyso-PC is removed from the medium, whereas others develop into gametocytes. There are certainly various additional factors involved in the sexual commitment of the parasites that are expressed at a certain time in the life cycle of the parasite or under certain conditions that are exclusively determined by the host. For example, the fact that the levels of some lyso-PC molecular species significantly decrease during the pyrogenic peak is one important host condition that needs to be examined in conjunction with parasite development and gene expression (97, 98).

The finding that lyso-PC plays an important role in parasite development, differentiation, and transmission makes the metabolic pathways and the involved enzymes very attractive targets for antimalarial drugs. A promising enzyme candidate might be the PfNSM, a lyso-PC–dependent phospholipase C (SM/LCPL–phospholipase C), which catalyzes the hydrolysis of sphingomyelin and lysophospholipids (Fig. 1) (27, 84). Hanada and co-workers (84) previously reported that the treatment with scyphostatin, a mycelial extract of the fungus Trichophyton mollissima, inhibited enzyme activity (ID₅₀ = 3–5 μM) as well as the growth of P. falciparum (–7 μM) (99).

Targeting PE synthesis is another attractive avenue to fight malaria. An in vivo screen of inhibitors against PkPSD has been previously reported (29). The screen searched for compounds that inhibit growth of a yeast mutant that relies on malarial PSD activity for survival. 18 candidates were identified as PSD inhibitors, and 7-chloro-N-(4-ethoxyphenyl)-4-quinolinamine (7CPQA analog) (MMV007285) was chosen for further analysis because of the ready availability of precursors, ease of synthesis, and low toxicity toward mammalian cells. The drug has been shown to be effective against P. falciparum with IC₅₀ values ranging between 0.5 and 1 μM (85), and it has a selectivity index of >8 in vitro. 7CPQA, an analog of MMV007285, has been shown to prevent Plasmodium yoelii growth in mice at 30 mg kg⁻¹ following a daily oral administration for 3 consecutive days (29). 7CPQA further inhibits all stages of parasite intra-erythrocytic development.

In vitro inhibitor screens amenable for high-throughput screening for decarboxylase enzymes have been challenging due to a lack of suitable enzyme assays. The conventional PSD enzyme assay is based on measuring captured radioactive CO₂, which is released following decarboxylation of 14C-PS by a PSD
enzyme (100). Recently, a fluorescence assay based on the interaction of PE with the water-soluble distarylbenzene-bisaldehyde (DSB-3) has been developed to facilitate chemical screens for inhibitors of PSD enzymes. The assay is amenable to high-throughput screening and can measure PSD activity of recombinant enzymes as well as native activity from cell extracts and membrane preparations from bacteria and yeast (83). Efforts are underway to identify more potent inhibitors of the malarial PSD enzyme using this assay. Finally, targeting PS biosynthesis through inhibition of PfPSS is another opportunity to inhibit parasite growth. However, no promising antimalarial candidates have been reported so far.

In summary, the lipid metabolism of Plasmodium parasites is both complex and fascinating. Not all of the puzzle pieces have been identified, but the already emerging picture gives a very promising outlook of the future of antimalarial chemotherapy due to the uniqueness of some of the already identified factors.

Perspectives

Efforts needed to further advance knowledge about phospholipid metabolism in development, differentiation, and malaria therapy include the following.

• Genetic analysis and target validation of phospholipid biosynthesis genes using CRISPR-Cas9 knockout and conditional knockout strategies.

• Biochemical and genetic characterization of the transport and metabolism of lyso-PC and other phospholipid precursors.

• Identification and development of new classes of inhibitors that target essential steps in membrane biogenesis.

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