Recent insights into fatty acid acquisition and metabolism in malarial parasites

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Abstract

The malarial parasite has a tremendous requirement for fatty acids during the replicative stages that take place in the mammalian host. A series of recent papers, discussed below, have revealed some of the mechanisms employed by the parasite to meet these demands.

Introduction and context

Upon infection of the mammalian host, malarial parasites complete a phase of development and multiplication in hepatocytes prior to initiating infection of erythrocytes. Given the high availability of nutrients in both of these environments, early investigators postulated that fatty acids required for the generation of progeny parasites might be scavenged entirely from the host.

Indeed, biochemical studies on blood-stage Plasmodium malarial parasites first demonstrated the parasite’s proficiency at scavenging and modifying lipids obtained from the host [1]. Free fatty acids can be obtained directly from the serum or from sources such as high-density lipoprotein [2-5]. Within the parasite, scavenged phospholipids can be incorporated without modification [3,4]. Additionally, Plasmodium parasites can modify fatty acids as needed by elongating or desaturating the lipids and incorporating them into phospholipids, diacylglycerols and triacylglycerols [6,7]. The minimal lipid requirement of blood-stage Plasmodium parasites was determined to be a combination of palmitic acid and oleic or steric acid, which could fully replace the need for serum in culture [6,8].

Such biochemical analysis has not been possible for liver-stage parasites because of the difficulty in isolating the parasite from the host hepatocyte and the low rate of infection during this stage. However, early studies of malaria in genetically obese Zucker rats (fa/fa) gave the first indication that host cell lipids are important for Plasmodium liver-stage development [9]. These studies demonstrated that obese Zucker rats, which have hepatocytes with increased fatty acid synthesis, triglyceridemia, and triglyceride content, supported the growth of four times the number of liver-stage schizonts than their leaner counterparts. Additionally, 45 hours after infection, schizonts in the obese rats were twice the size of those in control mice.

While it is not surprising that Plasmodium parasites depend on host sources of fatty acids, the role of de novo fatty acid synthesis in generating lipids remained controversial until the discovery of the apicoplast [10,11]. The apicoplast is a non-photosynthetic plastid organelle that was likely derived from a secondary endosymbiotic event during Apicomplexan evolution. Analysis of the P. falciparum genome revealed the presence of the apicoplast-resident biosynthetic fatty acid synthesis type II (FAS-II) pathway, which is involved in de novo synthesis of fatty acids [12,13]. The FAS-II pathway is responsible for the elongation of fatty acids via the action of four distinct enzymes: FabG, FabZ, FabI, and FabB/F. The main precursor for this pathway is acetyl-CoA, which is converted from pyruvate via the
activity of pyruvate dehydrogenase (PDH) in the apicoplast [14]. In vitro studies reconstituting the FAS-II pathway of Plasmodium provided evidence that Plasmodium FAS-II enzymes predominantly generate C10-C14 fatty acids [15]. Clinical in vivo transcriptional data on blood-stage parasites suggest that the FAS-II pathway can be upregulated during conditions of starvation [16]. However, aside from this auxiliary function in times of stress, the contribution of the FAS-II pathway during blood-stage development appears to be minimal. FAS-II enzymes are transcribed at a very low level in blood-stage parasites [17]. Furthermore, blood-stage growth rates are not impaired by deletion of fabI (in the human pathogen P. falciparum) or the virulent rodent model parasite P. berghei), fabB/F, fabZ, or PDH subunits pdh-e1α or pdh-e3 (in the less virulent rodent parasite, P. yoelii strain 17XNL) [18-20].

**Major recent advances**

Recent evidence suggests that the liver stage of parasite development, in contrast with the blood stage, requires a functional FAS-II pathway. This was initially suggested by the relatively high expression of FAS-II genes during this stage [21]. Conclusive evidence supporting a role for de novo fatty acid synthesis in the liver was provided by the observation that ΔfabI (in P. berghei), ΔfabZ, ΔfabB/F, Δpdh-e1α, and Δpdh-e3 (in P. yoelii) parasites exhibited deficiencies late in liver-stage development. This included a slow rate of growth, abnormal nuclear division, lack of cytomere formation, and lack of expression of late-stage proteins such as merozoite surface protein-1 (MSP-1) [18-20]. MSP-1 is a glycosyl-phosphatidylglycerol (GPI)-anchored protein present on the plasma membrane of merozoites, the progeny parasites that develop during the liver stage. Because merozoites are required to initiate blood-stage infection, it was expected that FAS-II-deficient parasites might not progress from liver to blood stage. Indeed, P. yoelii ΔfabB/F, ΔfabZ, Δpdh-e1α, and Δpdh-e3 liver-stage parasites were fully attenuated in their ability to produce a blood-stage infection in mice. P. berghei ΔfabI parasites exhibited a prepatent period that was twice that of wild-type parasites. Nevertheless, these did eventually lead to blood-stage infection. These differences in attenuation may be due to differences in virulence between the two species, which might permit clearance of low numbers of blood-stage forms of the less virulent P. yoelii parasites by the host immune response.

A screen of host cell proteins that might facilitate lipid scavenging by the parasite during the liver stage has pointed toward the surface-expressed cholesterol ester receptor, scavenger receptor class B type 1 (SR-BI) [22]. A decrease or complete loss of SR-BI surface availability via treatment with siRNA (short interfering RNA), antibodies, drugs, or gene deletion results in a reduction in parasite invasion and growth in hepatocytes [22,23]. The role of SR-BI expression in invasion and growth was shown to be multifactorial. SR-BI expression results in increased cell cholesterol levels. Together, SR-BI and cholesterol work synergistically to increase cell surface levels of CD81, particularly at tetraspanin-enriched microdomains [23,24]. This localization of CD81 at tetraspanin-enriched microdomains facilitates invasion by P. yoelii and P. falciparum sporozoites [23-25]. In addition, SR-BI expression results in upregulated liver fatty acid-binding protein (L-FABP) expression [23]. L-FABP is a liver-specific cytosolic fatty acid-binding protein responsible for transporting fatty acids. Presumably, L-FABP delivers host cell lipids to the growing parasite, supporting the metabolic needs of the rapidly growing parasite. The discovery that L-FABP binds the Plasmodium parasite protein UIS3 supports this hypothesis [25,26]. UIS3 is an essential liver-stage-specific parasite protein that is localized at the parasitophorous vacuole, a phospholipid bilayer interface that separates the parasite and the host cell [26,27]. The ability of UIS3 to co-crystallize with phosphatidylethanolamine suggests that UIS3 may cooperate with L-FABP to obtain phospholipids from the host cell [28].

**Future directions**

Many questions remain to be answered with regard to fatty acid metabolism in the Plasmodium liver stage. Identification of the fatty acids synthesized during the liver stage will provide insight into the minimal requirements for development during this stage. In addition, an understanding of the respective contribution of the scavenging and de novo synthesis pathways in parasite growth would facilitate the development of better strategies targeting the parasite during this stage.

While most scavenged and synthesized lipids are likely incorporated into the membranes of the rapidly growing parasite, additional roles for fatty acids remain to be elucidated. For example, studies have yet to identify the source of fatty acids that are incorporated into GPI, which anchors numerous proteins, including MSP-1, to the merozoite plasma membrane. The low expression of MSP-1 in fab knockout parasites suggests that the FAS-II pathway may act together with elongases and desaturases to generate the lipid anchor for some surface proteins, including MSP-1 [29]. Alternatively, the FAS-II pathway activity may provide a signal for progression to the merozoite formation phase of intrahepatic development. The specific upregulation of the FAS-II pathway during the liver stage may allow the parasite to synthesize its own...
lipids to supplement those provided by the host hepatocyte during a particularly explosive period of growth in the Plasmodium life cycle. This notion is supported by the decrease in growth rate of fabl, fabB/F, fabZ, pdh-e1a, and pdh-e3 knockout parasites in the liver. However, the parasite’s ability to multiply efficiently in the mosquito host suggests that the FAS-II pathway may be involved in processes unique to hepatic-stage development.

With respect to lipid scavenging, the mechanism of lipid import following association with UIS3 must be further examined. Presumably, parasite-encoded proteins that associate with UIS3 facilitate lipid incorporation into the membrane of the parasitophorous vacuole. However, the mechanisms of lipid import into the parasite remain unknown. Plasmodium fatty acid-binding proteins have not been characterized. A family of acyl-CoA synthetase homologues present in the P. falciparum genome may serve this function.

Both the lipid scavenging and lipid synthesis pathways offer opportunities for the development of antimalarials. Small molecules that interfere with the interaction of UIS3 with either L-FABP or phospholipids may abrogate infection in the liver. Genetically attenuated parasites deficient in de novo synthesis of fatty acids via FAS-II gene deletion, and rendered unable to import fatty acids from the host hepatocyte, may serve as effective pre-erythrocytic-stage whole-cell vaccines, analogous to the idea of using genetically attenuated sporozoite vaccines. Both strategies could protect the host from the debilitating symptoms that can arise following successful production of merozoites in the liver. Elucidating stage-specific requirements for import, synthesis, and utilization of fatty acids and lipids in malarial parasites is both of considerable promise for developing novel antimalarial intervention strategies and of fundamental interest in understanding how these parasites are so successful in establishing infection in the human host.

Abbreviations
FAS-II, fatty acid synthesis type II; GPI, glycosylphosphatidylinositol; L-FABP, liver fatty acid-binding protein; MSP-1, merozoite surface protein-1; PDH, pyruvate dehydrogenase; SR-BI, scavenger receptor class B type 1.

Competing interests
The authors declare that they have no competing interests.

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