BIOLOGY REPORTS

Published: 25 March 2010

Recent insights into fatty acid acquisition and metabolism in malarial parasites

Bamini Jayabalasingham[1], Robert Menard[2] and David A Fidock[1]*

Addresses: [1]Department of Microbiology & Immunology, College of Physicians and Surgeons, Columbia University, Room 1502, Hammer HSC, 701 W. 168th Street, New York, NY 10032, USA; [2]Institut Pasteur, Unité de Biologie et Génétique du Paludisme, 28 rue du Dr Roux, Paris cedex 15, Paris 75724, France

* Corresponding author: David A Fidock (df2260@columbia.edu)

F1000 Biology Reports 2010, **2**:24 (doi:10.3410/B2-24)

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 tri-acylglycerols [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 C_{10}-C_{14} 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-e1*α 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 Δ*fabI* (in *P. berghei*), Δ*fabZ*, Δ*fabB/F*, Δ*pdh-e1*α, and Δ*pdh-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 glycosylphosphatidylinositol (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* Δ*fabB/F*, Δ*fabZ*, Δ*pdh-e1*α, and Δ*pdh-e3* liver-stage parasites were fully attenuated in their ability to produce a blood-stage infection in mice. *P. berghei* Δ*fabI* 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 *fabI*, *fabB/F*, *fabZ*, *pdh-e1*α, 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.

Acknowledgments

Funding for this work was provided in part by a transformative R01 (AI085584) from the National Institutes of Health to DAF.

References

1. Vial HJ, Thuet MJ, Philippot JR: **Phospholipid biosynthesis in synchronous *Plasmodium falciparum* cultures.** *J Protozool* 1982, **29:**258-63.
2. Grellier P, Rigomier D, Clavey V, Fruchart JC, Schrevel J: **Lipid traffic between high density lipoproteins and *Plasmodium falciparum*-infected red blood cells.** *J Cell Biol* 1991, **112:**267-77.
3. Krishnegowda G, Gowda DC: **Intraerythrocytic *Plasmodium falciparum* incorporates extraneous fatty acids to its lipids without any structural modification.** *Mol Biochem Parasitol* 2003, **132:**55-8.
4. Moll GN, Vial HJ, Ancelin ML, Op den Kamp JAF, Roelofsen B, van Deenen LLM: **Phospholipid uptake by *Plasmodium knowlesi* infected erythrocytes.** *FEBS Letters* 1988, **232:**341-6.
5. Ofulla AV, Okoye VC, Khan B, Githure JI, Roberts CR, Johnson AJ, Martin SK: **Cultivation of *Plasmodium falciparum* parasites in a serum-free medium.** *Am J Trop Med Hyg* 1993, **49:**335-40.
6. Mi-Ichi F, Kita K, Mitamura T: **Intraerythrocytic *Plasmodium falciparum* utilize a broad range of serum-derived fatty acids with limited modification for their growth.** *Parasitology* 2006, **133:**399-410.
7. Palacpac NMQ, Hiramine Y, Mi-ichi F, Torii M, Kita K, Hiramatsu R, Horii T, Mitamura T: **Developmental-stage-specific triacylglycerol biosynthesis, degradation and trafficking as lipid bodies in *Plasmodium falciparum*-infected erythrocytes.** *J Cell Sci* 2004, **117:**1469-80.
8. Mi-Ichi F, Kano S, Mitamura T: **Oleic acid is indispensable for intraerythrocytic proliferation of *Plasmodium falciparum*.** *Parasitology* 2007, **134:**1671-7.
9. Lombard M-N, Bazin R, Durand D, Beauge F, Baccam D, Milgen F, Landau I: **Rodent *Plasmodium* development in livers of genetically obese Zucker rats (fa/fa).** *Eur J Protistol* 1998, **34:**78-81.
10. Kohler S, Delwiche CF, Denny PW, Tilney LG, Webster P, Wilson RJM, Palmer JD, Roos DS: **A plastid of probable green algal origin in Apicomplexan parasites.** *Science* 1997, **275:**1485-9.
11. McFadden GI, Reith ME, Munholland J, Lang-Unnasch N: **Plastid in human parasites.** *Nature* 1996, **381:**482.
12. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan M-S, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DMA, *et al.*: **Genome sequence of the human malaria parasite *Plasmodium falciparum*.** *Nature* 2002, **419:**498-511.

    f1000 Factor 6.0 *Must Read*
    Evaluated by Carol Sibley 16 Oct 2002

13. Ralph SA, van Dooren GG, Waller RF, Crawford MJ, Fraunholz MJ, Foth BJ, Tonkin CJ, Roos DS, McFadden GI: **Tropical infectious diseases: metabolic maps and functions of the *Plasmodium falciparum* apicoplast.** *Nat Rev Microbiol* 2004, **2:**203-16.
14. Foth B, Stimmler LM, Handman E, Crabb B, Hodder AN, McFadden G: **The malaria parasite *Plasmodium falciparum* has only one pyruvate dehydrogenase complex, which is located in the apicoplast.** *Mol Microbiol* 2005, **55:**39-53.

    f1000 Factor 6.0 *Must Read*
    Evaluated by Jurgen Soll 19 Jan 2005

15. Sharma S, Sharma SK, Modak R, Karmodiya K, Surolia N, Surolia A: **Mass spectrometry-based systems approach for identification of inhibitors of *Plasmodium falciparum* fatty acid synthase.** *Antimicrob Agents Chemother* 2007, **51:**2552-8.
16. Daily JP, Scanfeld D, Pochet N, Le Roch K, Plouffe D, Kamal M, Sarr O, Mboup S, Ndir O, Wypij D, Levasseur K, Thomas E, Tamayo P, Dong C, Zhou Y, Lander ES, Ndiaye D, Wirth D, Winzeler EA, Mesirov JP, Regev A: **Distinct physiological states of**

Plasmodium falciparum **in malaria-infected patients.** *Nature* 2007, **450:**1091-5.

f1000 Factor 9.0 *Exceptional*
Evaluated by Roberto Docampo 04 Jan 2008

17. Bozdech Z, Llinas M, Pulliam BL, Wong ED, Zhu J, DeRisi JL: **The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*.** *PLoS Biol* 2003, **1:**e5.

f1000 Factor 9.0 *Exceptional*
Evaluated by Emanuela Handman 20 Jan 2004

18. Yu M, Kumar TR, Nkrumah LJ, Coppi A, Retzlaff S, Li CD, Kelly BJ, Moura PA, Lakshmanan V, Freundlich JS, Valderramos JC, Vilcheze C, Siedner M, Tsai JH, Falkard B, Sidhu AB, Purcell L, Gratraud P, Kremer L, Waters A, Schiehser G, Jacobus DP, Janse C, Ager A, Jacobs WR, Sacchettini JC, Heussler V, Sinnis P, Fidock DA: **The fatty acid biosynthesis enzyme FabI plays a key role in the development of liver-stage malarial parasites.** *Cell Host Microbe* 2008, **4:**567-78.

f1000 Factor 6.4 *Must Read*
Evaluated by Frank Seeber 14 Jan 2009, Matthew Bogyo 12 Nov 2009

19. Vaughan AM, O'Neill MT, Tarun AS, Camargo N, Phuong TM, Aly ASI, Cowman AF, Kappe SHI: **Type II fatty acid synthesis is essential only for malaria parasite late liver stage development.** *Cell Microbiol* 2009, **11:**506-20.

20. Pei Y, Tarun AS, Vaughan AM, Herman RW, Soliman JMB, Erickson-Wayman A, Kappe SH: ***Plasmodium*** **pyruvate dehydrogenase activity is only essential for the parasite's progression from liver infection to blood infection.** *Mol Microbiol* 2010, [Epub ahead of print].

21. Tarun AS, Peng X, Dumpit RF, Ogata Y, Silva-Rivera H, Camargo N, Daly TM, Bergman LW, Kappe SH: **A combined transcriptome and proteome survey of malaria parasite liver stages.** *Proc Natl Acad Sci U S A* 2008, **105:**305-10.

f1000 Factor 6.0 *Must Read*
Evaluated by Paul Roepe 10 Jan 2008

22. Rodrigues CD, Hannus M, Prudencio M, Martin C, Goncalves LA, Portugal S, Epiphanio S, Akinc A, Hadwiger P, Jahn-Hofmann K, Rohl I, van Gemert GJ, Franetich J, Luty AJ, Sauerwein R, Mazier D, Koteliansky V, Vornlocher HP, Echeverri CJ, Mota MM: **Host scavenger receptor SR-BI plays a dual role in the establishment of malaria parasite liver infection.** *Cell Host Microbe* 2008, **4:**271-82.

23. Yalaoui S, Huby T, Franetich J, Gego A, Rametti A, Moreau M, Collet X, Siau A, van Gemert GJ, Sauerwein RW, Luty AJ, Vaillant JC, Hannoun L, Chapman J, Mazier D, Froissard P: **Scavenger receptor BI boosts hepatocyte permissiveness to *Plasmodium* infection.** *Cell Host Microbe* 2008, **4:**283-92.

24. Silvie O, Charrin S, Billard M, Franetich J-F, Clark KL, van Gemert G-J, Sauerwein RW, Dautry F, Boucheix C, Mazier D, Rubinstein E: **Cholesterol contributes to the organization of tetraspanin-enriched microdomains and to CD81-dependent infection by malaria sporozoites.** *J Cell Sci* 2006, **119:**1992-2002.

25. Silvie O, Rubinstein E, Franetich J, Prenant M, Belnoue E, Renia L, Hannoun L, Eling W, Levy S, Boucheix C, Mazier D: **Hepatocyte CD81 is required for *Plasmodium falciparum* and *Plasmodium yoelii* sporozoite infectivity.** *Nat Med* 2003, **9:**93-6.

f1000 Factor 6.0 *Must Read*
Evaluated by Dominique Soldati 14 Jan 2003

26. Mikolajczak SA, Jacobs-Lorena V, MacKellar DC, Camargo N, Kappe SH: **L-FABP is a critical host factor for successful malaria liver stage development.** *Int J Parasitol* 2007, **37:**483-9.

27. Mueller AK, Labaied M, Kappe SH, Matuschewski K: **Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine.** *Nature* 2005, **433:**164-7.

f1000 Factor 8.3 *Exceptional*
Evaluated by Rino Rappuoli 21 Jan 2005, Ulf Pettersson 24 Jan 2005, Stefan Kaufmann 01 Feb 2005, Ricardo Gazzinelli 15 Feb 2005

28. Sharma A, Yogavel M, Akhouri RR, Gill J, Sharma A: **Crystal structure of soluble domain of malaria sporozoite protein UIS3 in complex with lipid.** *J Biol Chem* 2008, **283:**24077-88.

f1000 Factor 3.0 *Recommended*
Evaluated by Rajesh Gokhale 23 Jul 2008

29. Gratraud P, Huws E, Falkard B, Adjalley S, Fidock DA, Berry L, Jacobs WR, Baird MS, Vial H, Kremer L: **Oleic acid biosynthesis in *Plasmodium falciparum*: characterization of the stearoyl-CoA desaturase and investigation as a potential therapeutic target.** *PLoS ONE* 2009, **4:**e6889.