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Active migration and passive transport of malaria parasites

Ross G. Douglas[1], Rogerio Amino[2], Photini Sinnis[3], and Freddy Frischknecht[1]

[1] Integrative Parasitology, Center for Infectious Diseases, University of Heidelberg Medical School, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany
[2] Unité de Biologie et Génétique du Paludisme, Département Parasites et Insectes Vecteurs, Institut Pasteur, 25–28 Rue du Dr Roux, 75015 Paris, France
[3] Johns Hopkins Malaria Research Institute, Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

Malaria parasites undergo a complex life cycle between their hosts and vectors. During this cycle the parasites invade different types of cells, migrate across barriers, and transfer from one host to another. Recent literature hints at a misunderstanding of the difference between active, parasite-driven migration and passive, circulation-driven movement of the parasite or parasite-infected cells in the various bodily fluids of mosquito and mammalian hosts. Because both active migration and passive transport could be targeted in different ways to interfere with the parasite, a distinction between the two ways the parasite uses to get from one location to another is essential. We discuss the two types of motion needed for parasite dissemination and elaborate on how they could be targeted by future vaccines or drugs.

Of sporozoites and skin: the parasite's journey to establish infection in the vertebrate host

A recent review on drug discovery in malaria rightly highlighted the need to block all stages of the parasite to achieve disease elimination [1]. Indeed, the complexity of the parasite life cycle, as this review points out, provides many challenges and opportunities for drug and vaccine design. Nonetheless, the authors miss what is likely an Achilles' heel of the parasite lifecycle in the mammalian host, namely the 'skin phase' [2,3]. This omission of the parasite's obligatory step in the skin can be found surprisingly often in the malaria research and clinical literature [1,4–8]. These reports mention that human infection begins with the transmission of sporozoites into the bloodstream during the bite of an infected female *Anopheles* mosquito. This implies direct injection of parasites into the bloodstream but overlooks the fact that sporozoites are predominantly, if not completely, injected extravascularly into the skin, where they need to be motile to cross the dermis and enter into either blood or lymph vessels [9–15]. The omission of the skin phase is unfortunate because the necessity of active parasite migration in the skin has opened up new opportunities for stopping the parasites before they enter the bloodstream, and skin migration may be an excellent drug and vaccine target. Indeed, sporozoite mutants with motility defects are significantly more attenuated after inoculation into the skin compared to after intravenous inoculation [16–19]. Similarly, parasite mutants defective in cell traversal activity, the sporozoite capacity to wound and transmigrate a host cell, also show impaired progression in the skin [20–22]. Furthermore, antibodies that immobilize sporozoites have a clear effect on sporozoite motility in the dermis [23–25]. The majority of the time that sporozoites are in an extracellular environment is spent in the dermis, where they are likely to be much more vulnerable to antibodies and possibly drugs, compared to the blood circulation where they only spend minutes, and the liver where they rapidly find and invade hepatocytes (Table 1) [15,26–29].

Statements such as 'motile sporozoites migrate to the liver and invade hepatocytes' [1] could lead to the impression that sporozoites use their capacity for motility to actively migrate in the blood to the liver. However, once sporozoites are in the blood they are carried by the blood flow until they arrest on the endothelium of the liver. In the liver, sporozoites actively migrate again to cross the endothelial barrier and enter hepatocytes [30,31]. The capacity to actively migrate is clearly important for sporozoite entry into hepatocytes [19,32]. However, fast and robust motility is only needed in the skin because diminished motility still allows sporozoites to effectively enter the liver if they are injected by syringe directly into the bloodstream [16–19,21,33]. This suggests that the malaria parasite has evolved a high speed specifically to cross the dermis, a finding that might well be important in intervention considerations that target their motility. Indeed, a recent study suggests that antibodies that block motility have a greater effect in the dermis (on sporozoites inoculated by mosquito bites) than in the circulation (on sporozoites inoculated directly into the bloodstream) [23].

Nonetheless, the passively-moving sporozoites in the blood might be targeted to prevent them from reaching the liver, once it is known what proteins on the sporozoite

Corresponding author: Frischknecht, F. (freddy.frischknecht@med.uni-heidelberg.de).
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Table 1. Summary of active movement and passive transport of *Plasmodium* at different life cycle stages

Parasite stage	Organ/tissue/cell/dissemination route	Event (active/passive)	Estimated time for event
Sporozoite	Hemocoel	Release from oocyst, passive transport in hemolymph	Unknown; possibly 1 minute to 1 day
	Salivary gland	Invasion of glands	Unknown; possibly 1–10 minutes
	Proboscis	Transport from mosquito to skin with flow of saliva	~1 second
	Skin	Motility through tissue and invasion of vasculature	~10–100 minutes
	Lymphatic system	Transport in lymph vessels, active movement in node	~10–100 minutes
	Bloodstream	Transport to liver	1–10 minutes
	Liver	Cell traversal and hepatocyte invasion	~1 minute
Merozoite	Merosomes	Bud off from infected hepatocytes, passive transport in blood	~1–10 minutes
	Erythrocytes in bloodstream	Passive transport in blood after merosome rupture, invasion of RBC; following RBC rupture, momentary passive transport in blood before actively invading another RBC	~1 minute
Gamete	Mosquito midgut	Active swimming by male gamete to fertilize female	~1–10 minutes
Ookinete	Mosquito midgut peritrophic matrix and epithelia	Presumably passive transport until contact with peritrophic matrix; active motility to traverse midgut epithelia	~1–10 minutes

Active (red)/passive (blue).

surface mediate this process. Several interactions are already known, including those of the thrombospondin-related anonymous protein (TRAP) and circumsporozoite protein (CSP) with hepatic heparan sulfate proteoglycans [34,35]. Clearly, the sporozoite's journey from the point of inoculation to final entry into a hepatocyte is complex and requires many different proteins: while some interventions might target an actively-migrating sporozoite, others may only target sporozoite interactions with host factors once the sporozoite enters the bloodstream or becomes arrested in the liver. In addition, while some inhibitors may be specific for a particular phase of the journey, others may target sporozoites at all phases. Thus, the distinction between passive, circulation-driven movement and active, parasite-driven migration is not only of terminological value (Figure 1 and Table 1).

Sporozoites inoculated into the dermis also enter the lymphatic system. They can be seen migrating actively within the dermis, and upon entering lymph vessels are passively transported along with the lymph [3,10]. When arriving at the subcapsular sinus of lymph nodes, they appear to actively glide again and eventually encounter phagocytic cells that appear to clear the vast majority of parasites [10]. Because only few sporozoites are injected into the skin during natural bites, and only about 20% of these enter the lymphatic system [10,15], it is not clear if there is an immune response to these parasites – and if so whether it would be of a tolerogenic or activating nature [36,37]. However, during immunization regimes where sporozoites are used as live attenuated parasite vaccines, the large number of parasites arriving at the lymph node does elicit protective immune responses in mice [36,38]. Importantly, RTS,S, the only malaria vaccine candidate that has demonstrated any efficacy in Phase III trials, is a subunit vaccine based on CSP, and follow-up studies generally suggest that protection is correlated to antibody titers [39–41]. It is possible that these antibodies primarily target sporozoites in the skin, where they spend most of their time before invading hepatocytes. Again, it appears important to distinguish between passive transport within the lymphatic vessels, which would not be affected by antibodies, and active motility in the skin and lymph node. The latter could lead to fewer sporozoites arriving in the draining lymph node, and thus lead to an altered immune response during subsequent immunizations.

Sporozoite movements in the mosquito

Sporozoites must also travel in the mosquito, and this journey also often suffers from similar confusion between active migration and passive transport [42–44]. Sporozoites develop in oocysts in the mosquito midgut wall, and must exit into the hemocoel and go to the salivary gland. In the hemocoel, sporozoites are passively transported by the movement of the mosquito hemolymph and, although they are carried throughout the open circulatory system of the mosquito, they appear to preferentially recognize and invade salivary glands [16,32,45–48]. Similarly to the sporozoites that make it into the bloodstream, the parasites in the hemolymph have the capacity to actively migrate but do not do so in the open circulation of the mosquito. Only once sporozoites attach to the salivary glands do they need to move across the basal membrane and the acinar cells to accumulate in the salivary cavity of these glands [49–51].

Interestingly, once sporozoites have gained access to the salivary gland acinar cell, little forward motility can be observed. Most parasites move back-and-forth with little productive motility [51]. As the infected mosquito probes for blood it ejects sporozoites along with its saliva. Once again, the sporozoites are passively transported by the flow of mosquito saliva. Thus a single parasite stage undergoes several different 'movement transitions' (Figure 1 and Table 1).

Moving onto other life stages

Importantly, not only the sporozoite stage undergoes cycles of active migration and passive transport. After sporozoite invasion of the host liver, merozoites are formed by the

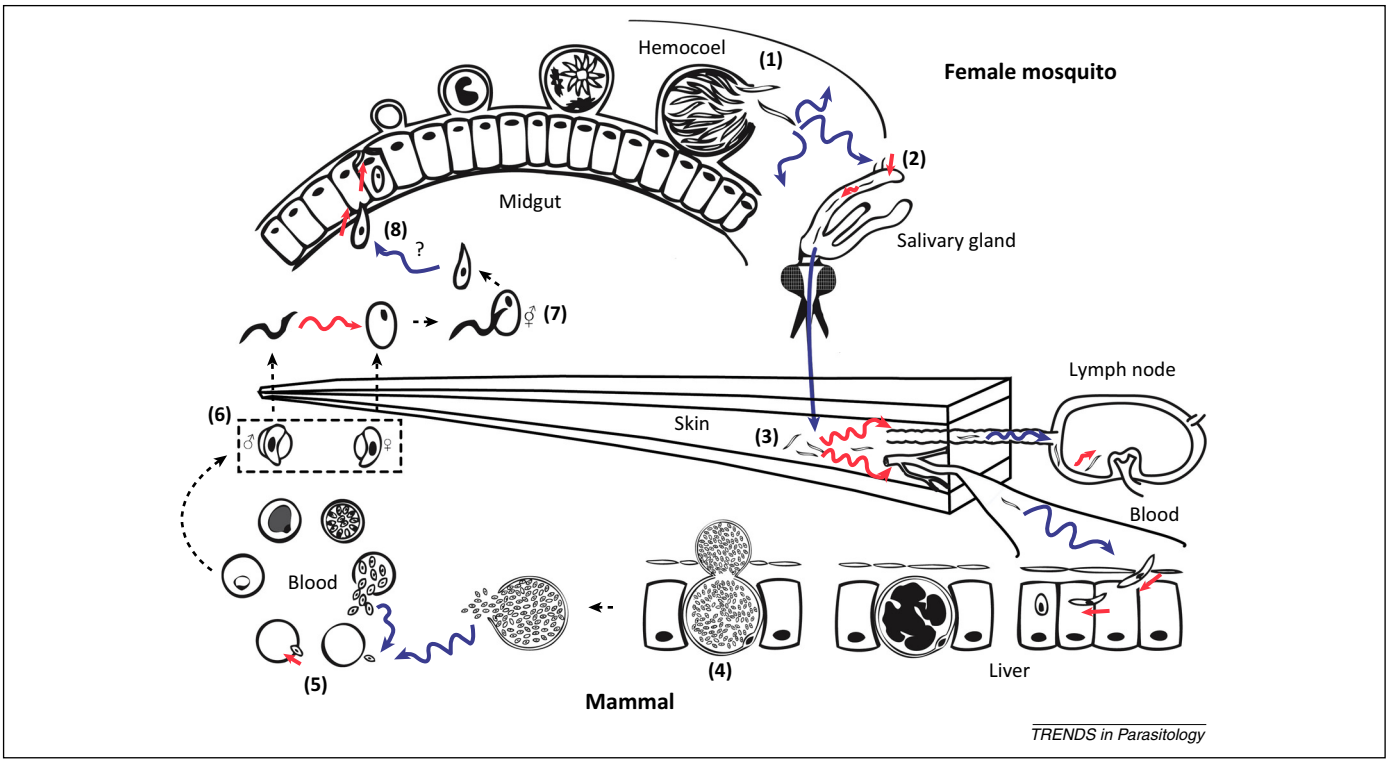

Figure 1. Active movement and passive transport transitions along the Plasmodium life cycle. Stages of active migration (red arrows) and passive transport (blue arrows). Sporozoites emerge from the oocyst **(1)** and are passively transported within the hemolymph before actively migrating into the salivary glands **(2)**. From there, they are passively ejected with the saliva and take up active motility again in the skin **(3)**. Once they enter either the lymph or blood, again they are passively transported to actively enter either the lymph node or the liver parenchyma. Sporozoites that have entered the liver differentiate within a hepatocyte; following the development of exo-erythrocytic forms, merosomes bud from the infected hepatocyte **(4)**. After membrane rupture, merosomes release hundreds to thousands of merozoites into the blood that briefly are carried by the blood before attaching to and actively invading erythrocytes **(5)**. Within the erythrocyte, schizogony results in more merozoites that then egress from cells. These merozoites are passively carried in the blood and, subsequently, actively invade nearby uninfected erythrocytes. Some merozoites actively invade and develop into male or female gametocytes that await transmission into the arthropod host **(6)**. In the mosquito, gamete maturation results in actively-moving male gametes that fuse with females to produce a zygote **(7)**. Zygotes develop into motile ookinetes that can actively traverse the mosquito peritrophic matrix and midgut epithelia **(8)** to establish oocysts in the basal lamina of the insect host.

thousands in infected hepatocytes, and these were long thought to get into the bloodstream by simple rupture of the hepatocyte, although it was not clear how they could cross the endothelium to reach the bloodstream [52–54]. Active migration was one possibility, because merozoites possess the necessary gliding machinery; however, it was discovered that small bags of merozoites bud off as merosomes from the infected cells [55]. Merosomes rupture to release merozoites [56] that presumably are passively carried in the blood until they attach to and invade a red blood cell (Figure 1). In observed static conditions, merozoites enter red blood cells by actively migrating across a junction they establish between the two cells [57]. After invasion, growth, and schizogony, new merozoites are explosively released from the red blood cell [58], and are briefly transported again within the blood before attaching to and invading an uninfected blood cell (Figure 1 and Table 1). Attachment to and invasion of red blood cells are thought of as good targets for vaccines and possibly also drugs [59,60]. Several proteins involved in either or both processes are at different stages of subunit vaccine development with the hope that antibodies against these proteins could protect infected people by blocking the access of the parasite to the red blood cell [61–63]. Apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP1) are such candidates. The role of MSP1 (the major surface antigen of merozoites) in mediating initial attachment to target erythrocytes appears to be clear [64]. However, the role played by AMA1 is contested – initial data suggest a role in invasion whereas more recent data indicate a role in attachment [65,66]. For our discourse, understanding whether AMA1 functions in invasion or attachment will determine when antibodies need to act to prevent infection.

Not all merozoites that successfully invade proceed to undergo schizogony and produce more merozoites. A fraction of invaded parasites transform into male and female gametocytes – parasites under cell cycle arrest that are prepared for transmission back to the mosquito [67]. After gametogenesis, the male gamete actively swims within the bolus of the blood-meal in the mosquito stomach and fuses with the female gamete [68]. Transmission-blocking approaches can target multiple aspects: gamete maturation, the active motility of the male gamete, or the docking of the two cells [69–73]. Finally, after fertilization and zygote formation, a motile egg cell, the ookinete, forms and actively penetrates the peritrophic matrix surrounding the blood meal and the underlying epithelial cells of the mosquito midgut. Before attaching to the matrix, the ookinete may not actively move in the mosquito midgut because this environment provides little traction for gliding motility. However, once attached to the mosquito midgut, the ookinete actively moves across this cell layer [74] (Figure 1). Secreted or surface proteins

of the ookinetes that assist in traversal again could serve as targets for transmission-blocking antibodies or peptides, and thus prevent oocyst formation in the mosquito [75–77].

Opportunities in stopping parasite movement

The complexities of the *Plasmodium* life cycle provide many challenges in fully understanding how the parasite proliferates and disseminates, but also offer us multiple opportunities for intervention. Indeed, developing drug or vaccine combinations that could target multiple proteins and stages is desirable [1]. It is our intention in this article to point out that blocking the ability of the parasite to actively move and/or interact with host factors for invasion opens up both therapeutic and preventive opportunities. The skin serves as the first mammalian barrier for the sporozoite, and is thus the first site where preventive agents can be employed. The skin phase is a significant bottleneck for the parasite both in terms of numbers and exposure to antibody-mediated responses. Studies in mice have supported the idea that antibody-mediated responses can halt active movement and/or result in complement-mediated cytotoxicity of sporozoites in the skin to prevent infection [23–25,78]. Hence, while one could contemplate strategies to activate complement or immune cells that stop the passively transported parasites in the blood, it might also be sensible to develop strategies to block active migration. For example, if one targets a conserved motility mechanism by a drug, this could likewise block active migration of sporozoites, invasion of erythrocytes by merozoites, or ookinete motility [79]. There are indeed suggestions in the literature that low molecular weight molecules could be used to stop active parasite movement [80–86]. Perhaps potent and parasite-selective inhibitors could be developed for formulation in topical insect-repellant creams that would serve as a malaria prophylaxis strategy. Compound libraries such as those made available by Medicines for Malaria Venture could accelerate the discovery and development of such molecules [87].

Concluding remarks

The malaria parasite undergoes alternating phases of active and passive migration in different tissues during its life cycle. The life cycle can be subdivided into at least seven actively-motile stages and six stages in which it is passively carried by the mosquito or mammalian host, each presenting different opportunities as drug or vaccine targets. Appreciating the full complexity of this fascinating parasite would allow for more targeted therapeutic interventions.

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References

1 Flannery, E.L. *et al.* (2013) Antimalarial drug discovery – approaches and progress towards new medicines. *Nat. Rev. Microbiol.* 11, 849–862
2 Sinnis, P. and Zavala, F. (2012) The skin: where malaria infection and the host immune response begin. *Semin. Immunopathol.* 34, 787–792
3 Menard, R. *et al.* (2013) Looking under the skin: the first steps in malarial infection and immunity. *Nat. Rev. Microbiol.* 11, 701–712
4 Tilley, L. *et al.* (2011) The *Plasmodium falciparum*-infected red blood cell. *Int. J. Biochem. Cell Biol.* 43, 839–842
5 Collins, W.E. and Jeffery, G.M. (2007) *Plasmodium malariae*: parasite and disease. *Clin. Microbiol. Rev.* 20, 579–592
6 Haldar, K. *et al.* (2007) Malaria: mechanisms of erythrocytic infection and pathological correlates of severe disease. *Annu. Rev. Pathol.* 2, 217–249
7 Hall, J. and Ross, E. (2012) *Malaria: The Battle Against a Microscopic Killer*, EVIMalaR
8 Bousema, T. *et al.* (2014) Asymptomatic malaria infections: detectability, transmissibility and public health relevance. *Nat. Rev. Microbiol.* 12, 833–840
9 Kappe, S.H. *et al.* (2004) *Plasmodium* sporozoite molecular cell biology. *Annu. Rev. Cell Dev. Biol.* 20, 29–59
10 Amino, R. *et al.* (2006) Quantitative imaging of *Plasmodium* transmission from mosquito to mammal. *Nat. Med.* 12, 220–224
11 Sidjanski, S. and Vanderberg, J.P. (1997) Delayed migration of *Plasmodium* sporozoites from the mosquito bite site to the blood. *Am. J. Trop. Med. Hyg.* 57, 426–429
12 Matsuoka, H. *et al.* (2002) A rodent malaria, *Plasmodium berghei*, is experimentally transmitted to mice by merely probing of infective mosquito, Anopheles stephensi. *Parasitol. Int.* 51, 17–23
13 Medica, D.L. and Sinnis, P. (2005) Quantitative dynamics of *Plasmodium yoelii* sporozoite transmission by infected anopheline mosquitoes. *Infect. Immun.* 73, 4363–4369
14 Amino, R. *et al.* (2005) In vivo imaging of malaria parasites – recent advances and future directions. *Curr. Opin. Microbiol.* 8, 407–414
15 Yamauchi, L.M. *et al.* (2007) *Plasmodium* sporozoites trickle out of the injection site. *Cell. Microbiol.* 9, 1215–1222
16 Ejigiri, I. *et al.* (2012) Shedding of TRAP by a rhomboid protease from the malaria sporozoite surface is essential for gliding motility and sporozoite infectivity. *PLoS Pathog.* 8, e1002725
17 Volkmann, K. *et al.* (2012) The alveolin IMC1 h is required for normal ookinete and sporozoite motility behaviour and host colonisation in *Plasmodium berghei*. *PLoS ONE* 7, e41409
18 Hellmann, J.K. *et al.* (2011) Environmental constraints guide migration of malaria parasites during transmission. *PLoS Pathog.* 7, e1002080
19 Montagna, G.N. *et al.* (2012) Critical role for heat shock protein 20 (HSP20) in migration of malarial sporozoites. *J. Biol. Chem.* 287, 2410–2422
20 Amino, R. *et al.* (2008) Host cell traversal is important for progression of the malaria parasite through the dermis to the liver. *Cell Host Microbe* 3, 88–96
21 Moreira, C.K. *et al.* (2008) The *Plasmodium* TRAP/MIC2 family member, TRAP-like protein (TLP), is involved in tissue traversal by sporozoites. *Cell Microbiol.* 10, 1505–1516
22 Bhanot, P. *et al.* (2005) A surface phospholipase is involved in the migration of *Plasmodium* sporozoites through cells. *J. Biol. Chem.* 280, 6752–6760
23 Sack, B.K. *et al.* (2014) Model for in vivo assessment of humoral protection against malaria sporozoite challenge by passive transfer of monoclonal antibodies and immune serum. *Infect. Immun.* 82, 808–817
24 Vanderberg, J.P. and Frevert, U. (2004) Intravital microscopy demonstrating antibody-mediated immobilisation of *Plasmodium berghei* sporozoites injected into skin by mosquitoes. *Int. J. Parasitol.* 34, 991–996
25 Kebaier, C. *et al.* (2009) Kinetics of mosquito-injected *Plasmodium* sporozoites in mice: fewer sporozoites are injected into sporozoite-immunized mice. *PLoS Pathog.* 5, e1000399
26 Sinnis, P. *et al.* (1996) Remnant lipoproteins inhibit malaria sporozoite invasion of hepatocytes. *J. Exp. Med.* 184, 945–954
27 Cerami, C. *et al.* (1994) Rapid clearance of malaria circumsporozoite protein (CS) by hepatocytes. *J. Exp. Med.* 179, 695–701

28 Shin, S.C. *et al.* (1982) Direct infection of hepatocytes by sporozoites of *Plasmodium berghei*. *J. Protozool.* 29, 448–454
29 Vanderberg, J. *et al.* (2007) Assessment of antibody protection against malaria sporozoites must be done by mosquito injection of sporozoites. *Am. J. Pathol.* 171, 1405–1406
30 Frevert, U. *et al.* (2005) Intravital observation of *Plasmodium berghei* sporozoite infection of the liver. *PLoS Biol.* 3, e192
31 Tavares, J. *et al.* (2013) Role of host cell traversal by the malaria sporozoite during liver infection. *J. Exp. Med.* 210, 905–915
32 Sultan, A.A. *et al.* (1997) TRAP is necessary for gliding motility and infectivity of *Plasmodium* sporozoites. *Cell* 90, 511–522
33 Sato, Y. *et al.* (2014) *Plasmodium berghei* sporozoites acquire virulence and immunogenicity during mosquito hemocoel transit. *Infect. Immun.* 82, 1164–1172
34 Coppi, A. *et al.* (2007) Heparan sulfate proteoglycans provide a signal to *Plasmodium* sporozoites to stop migrating and productively invade host cells. *Cell Host Microbe* 2, 316–327
35 Pradel, G. *et al.* (2002) Proteoglycans mediate malaria sporozoite targeting to the liver. *Mol. Microbiol.* 45, 637–651
36 Chakravarty, S. *et al.* (2007) $CD8^+$ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes. *Nat. Med.* 13, 1035–1041
37 Guilbride, D.L. *et al.* (2010) Why functional pre-erythrocytic and bloodstage malaria vaccines fail: a meta-analysis of fully protective immunizations and novel immunological model. *PLoS ONE* 5, e10685
38 Obeid, M. *et al.* (2013) Skin-draining lymph node priming is sufficient to induce sterile immunity against pre-erythrocytic malaria. *EMBO Mol. Med.* 5, 250–263
39 Campo, J.J. *et al.* (2014) Duration of vaccine efficacy against malaria: 5th year of follow-up in children vaccinated with RTS, S/AS02 in Mozambique. *Vaccine* 32, 2209–2216
40 White, M.T. *et al.* (2014) A combined analysis of immunogenicity, antibody kinetics and vaccine efficacy from phase 2 trials of the RTS,S malaria vaccine. *BMC Med.* 12, 117
41 Arama, C. and Troye-Blomberg, M. (2014) The path of malaria vaccine development: challenges and perspectives. *J. Intern. Med.* 275, 456–466
42 Sreenivasamurthy, S.K. *et al.* (2013) A compendium of molecules involved in vector-pathogen interactions pertaining to malaria. *Malar. J.* 12, 216
43 Baton, L.A. *et al.* (2009) Genome-wide transcriptomic profiling of *Anopheles gambiae* hemocytes reveals pathogen-specific signatures upon bacterial challenge and Plasmodium berghei infection. *BMC Genomics* 10, 257
44 Gunn, A. and Pitt, S.J. (2012) *Parasitology: An Integrated Approach*, John Wiley & Sons
45 Kariu, T. *et al.* (2002) MAEBL is essential for malarial sporozoite infection of the mosquito salivary gland. *J. Exp. Med.* 195, 1317–1323
46 Golenda, C.F. *et al.* (1990) The distribution of circumsporozoite protein (CS) in *Anopheles stephensi* mosquitoes infected with *Plasmodium falciparum* malaria. *J. Histochem. Cytochem.* 38, 475–481
47 Frischknecht, F. *et al.* (2006) Using green fluorescent malaria parasites to screen for permissive vector mosquitoes. *Malar. J.* 5, 23
48 Akaki, M. and Dvorak, J.A. (2005) A chemotactic response facilitates mosquito salivary gland infection by malaria sporozoites. *J. Exp. Biol.* 208, 3211–3218
49 Sterling, C.R. *et al.* (1973) The passage of *Plasmodium berghei* sporozoites through the salivary glands of *Anopheles stephensi*: an electron microscope study. *J. Parasitol.* 59, 593–605
50 Pimenta, P.F. *et al.* (1994) The journey of malaria sporozoites in the mosquito salivary gland. *J. Eukaryot. Microbiol.* 41, 608–624
51 Frischknecht, F. *et al.* (2004) Imaging movement of malaria parasites during transmission by *Anopheles* mosquitoes. *Cell. Microbiol.* 6, 687–694
52 Meis, J.F. *et al.* (1985) Fine structure of exoerythrocytic merozoite formation of *Plasmodium berghei* in rat liver. *J. Protozool.* 32, 694–699
53 Sturm, A. and Heussler, V. (2007) Live and let die: manipulation of host hepatocytes by exoerythrocytic *Plasmodium* parasites. *Med. Microbiol. Immunol.* 196, 127–133
54 Graewe, S. *et al.* (2012) Chronicle of a death foretold: *Plasmodium* liver stage parasites decide on the fate of the host cell. *FEMS Microbiol. Rev.* 36, 111–130
55 Sturm, A. *et al.* (2006) Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. *Science* 313, 1287–1290
56 Baer, K. *et al.* (2007) Release of hepatic *Plasmodium yoelii* merozoites into the pulmonary microvasculature. *PLoS Pathog.* 3, e171
57 Baum, J. *et al.* (2008) Host-cell invasion by malaria parasites: insights from Plasmodium and Toxoplasma. *Trends Parasitol.* 24, 557–563
58 Abkarian, M. *et al.* (2011) A novel mechanism for egress of malarial parasites from red blood cells. *Blood* 117, 4118–4124
59 Rodriguez, L.E. *et al.* (2008) Intimate molecular interactions of *P. falciparum* merozoite proteins involved in invasion of red blood cells and their implications for vaccine design. *Chem. Rev.* 108, 3656–3705
60 Chandramohanadas, R. *et al.* (2014) Small molecule targeting malaria merozoite surface protein-1 (MSP-1) prevents host invasion of divergent plasmodial species. *J. Infect. Dis.* 210, 1616–1626
61 O'Donnell, R.A. *et al.* (2001) Antibodies against merozoite surface protein (MSP)-1(19) are a major component of the invasion-inhibitory response in individuals immune to malaria. *J. Exp. Med.* 193, 1403–1412
62 Barry, A.E. and Arnott, A. (2014) Strategies for designing and monitoring malaria vaccines targeting diverse antigens. *Front. Immunol.* 5, 359
63 Wright, G.J. and Rayner, J.C. (2014) *Plasmodium falciparum* erythrocyte invasion: combining function with immune evasion. *PLoS Pathog.* 10, e1003943
64 Kadekoppala, M. and Holder, A.A. (2010) Merozoite surface proteins of the malaria parasite: the MSP1 complex and the MSP7 family. *Int. J. Parasitol.* 40, 1155–1161
65 Giovannini, D. *et al.* (2011) Independent roles of apical membrane antigen 1 and rhoptry neck proteins during host cell invasion by apicomplexa. *Cell Host Microbe* 10, 591–602
66 Bargieri, D.Y. *et al.* (2013) Apical membrane antigen 1 mediates apicomplexan parasite attachment but is dispensable for host cell invasion. *Nat. Commun.* 4, 2552
67 Kuehn, A. and Pradel, G. (2010) The coming-out of malaria gametocytes. *J. Biomed. Biotechnol.* 2010, 976827
68 Sinden, R.E. and Croll, N.A. (1975) Cytology and kinetics of microgametogenesis and fertilization in *Plasmodium yoelii nigeriensis*. *Parasitology* 70, 53–65
69 Gwadz, R.W. (1976) Successful immunization against the sexual stages of *Plasmodium gallinaceum*. *Science* 193, 1150–1151
70 Carter, R. *et al.* (1979) *Plasmodium gallinaceum*: transmission-blocking immunity in chickens. II. The effect of antigamete antibodies in vitro and in vivo and their elaboration during infection. *Exp. Parasitol.* 47, 194–208
71 Healer, J. *et al.* (1999) Transmission-blocking immunity to *Plasmodium falciparum* in malaria-immune individuals is associated with antibodies to the gamete surface protein Pfs230. *Parasitology* 119, 425–433
72 Hirai, M. and Mori, T. (2010) Fertilization is a novel attacking site for the transmission blocking of malaria parasites. *Acta Trop.* 114, 157–161
73 Ojo, K.K. *et al.* (2012) Transmission of malaria to mosquitoes blocked by bumped kinase inhibitors. *J. Clin. Invest.* 122, 2301–2305
74 Angrisano, F. *et al.* (2012) Malaria parasite colonisation of the mosquito midgut – placing the *Plasmodium* ookinete centre stage. *Int. J. Parasitol.* 42, 519–527
75 Barr, P.J. *et al.* (1991) Recombinant Pfs25 protein of *Plasmodium* falciparum elicits malaria transmission-blocking immunity in experimental animals. *J. Exp. Med.* 174, 1203–1208
76 Shahabuddin, M. *et al.* (1993) Transmission-blocking activity of a chitinase inhibitor and activation of malarial parasite chitinase by mosquito protease. *Proc. Natl. Acad. Sci. U.S.A.* 90, 4266–4270
77 Birkett, A.J. *et al.* (2013) Malaria vaccine R&D in the Decade of Vaccines: breakthroughs, challenges and opportunities. *Vaccine* 31 (Suppl. 2), B233–B243
78 Yilmaz, B. *et al.* (2014) Gut microbiota elicits a protective immune response against malaria transmission. *Cell* 159, 1277–1289
79 Baum, J. *et al.* (2006) Regulation of apicomplexan actin-based motility. *Nat. Rev. Microbiol.* 4, 621–628

80 Hellmann, J.K. *et al.* (2010) Synergistic and additive effects of epigallocatechin gallate and digitonin on *Plasmodium* sporozoite survival and motility. *PLoS ONE* 5, e8682
81 Lentz, C.S. *et al.* (2015) In vitro activity of wALADin benzimidazoles against different life cycle stages of *Plasmodium* parasites. *Antimicrob. Agents Chemother.* 59, 654–658
82 Liehl, P. *et al.* (2010) Phosphothioate oligodeoxynucleotides inhibit *Plasmodium* sporozoite gliding motility. *Cell. Microbiol.* 12, 506–515
83 Miller, L.H. *et al.* (1979) Interaction between cytochalasin B-treated malarial parasites and erythrocytes. Attachment and junction formation. *J. Exp. Med.* 149, 172–184
84 Siden-Kiamos, I. *et al.* (2006) Involvement of actin and myosins in *Plasmodium berghei* ookinete motility. *Mol. Biochem. Parasitol.* 150, 308–317
85 Munter, S. *et al.* (2009) *Plasmodium* sporozoite motility is modulated by the turnover of discrete adhesion sites. *Cell Host Microbe* 6, 551–562
86 Mizuno, Y. *et al.* (2002) Effect of jasplakinolide on the growth, invasion, and actin cytoskeleton of *Plasmodium falciparum*. *Parasitol. Res.* 88, 844–848
87 Spangenberg, T. *et al.* (2013) The open access malaria box: a drug discovery catalyst for neglected diseases. *PLoS ONE* 8, e62906