Minireview

Malaria sporozoite proteome leaves a trail

Marissa Vignali*, Cate Speake*† and Patrick E Duffy*†

Addresses: *Malaria Program, Seattle Biomedical Research Institute, Seattle, Washington 98109, USA. †Department of Global Health, University of Washington, Seattle, Washington 98195, USA.

Correspondence: Patrick E Duffy. Email: patrick.duffy@sbri.org

Published: 21 April 2009

Genome Biology 2009, **10:**216 (doi:10.1186/gb-2009-10-4-216)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2009/10/4/216

Abstract

The malaria parasite sporozoite proteome changes during maturation, revealing proteins specifically expressed in the stage that infects the human host.

Every year, malaria causes an estimated 250-500 million clinical cases worldwide, resulting in nearly a million deaths. Despite growing awareness and substantial public-health efforts aimed at reducing the global malaria burden, more than a billion people remain at risk of contracting the disease, and control remains elusive: there is currently no licensed vaccine, and resistance has developed to nearly all available antimalarial drugs [1]. A study published recently by Lasonder and colleagues in *PLoS Pathogens* [2] applies a proteomics-based reverse genetics approach to identify proteins expressed specifically in the stages that immediately precede human infection, resulting in the identification of new candidates for drugs and vaccines that might prevent infection by *Plasmodium falciparum*.

Malaria is caused by *Plasmodium* parasites, which have a complex life cycle (Figure 1). Sexual parasite forms, or gametocytes, that are generated at low frequency during the blood stage are taken up in a mosquito bloodmeal. Gamete fertilization occurs in the mosquito midgut, and the resulting zygote develops into an ookinete, which penetrates the wall of the midgut and becomes an oocyst. The parasite then reproduces asexually within this membrane-bound form, generating thousands of oocyst-derived sporozoites (ODS). These are released into the mosquito hemocoel upon rupture of the oocyst, and subsequently invade the salivary glands. When the infected mosquito bites a new mammalian host, salivary gland sporozoites (SGS) are injected, which migrate from the skin into blood vessels and then circulate to the liver. Hepatocyte invasion initiates the liver stage. Extensive differentiation and multiplication ensues, with each liver-stage parasite yielding tens of thousands of merozoites. Asexual reproduction then occurs within erythrocytes, leading to the clinical symptoms that occur during the blood stage of the disease. When these cells rupture, new waves of merozoites are released into the human bloodstream to invade fresh erythrocytes.

In recent years, high-throughput approaches to identifying novel drug and vaccine targets have been made possible by genome sequencing of *Plasmodium* species. The genomes of *P. falciparum*, the deadliest human parasite [3], and that of a model rodent species, *P. yoelii* [4], were published in 2002. These were followed by the genomes of rodent species *P. berghei* and *P. chabaudi* in 2005 [5], and those of *P. vivax* and *P. knowlesi*, two other species that infect humans, in 2008 [6,7]. These studies subsequently enabled several transcriptome and proteome analyses (reviewed in [8]). Most of these efforts were focused on *P. falciparum* blood-stage parasites that can be cultured *in vitro*, and on rodent parasite model species. Because of technical difficulties in working with liver-stage forms, our knowledge of the genes and proteins expressed during this stage is less complete. However, intervention strategies aimed at pre-erythrocytic stages (sporozoite and liver-stage parasites) are considered promising because of the low number of parasites transmitted by the mosquito [9] and the fact that sterile immunity against the pre-erythrocytic forms of both human and rodent parasites can be induced by immunization with attenuated parasites [10,11]. A better understanding of mosquito and liver-stage parasites is necessary to develop and improve on pre-erythrocytic stage interventions. Functional genomic studies such as those by Lasonder *et al.* [2] will further our knowledge of this important period of malaria pathogenesis.

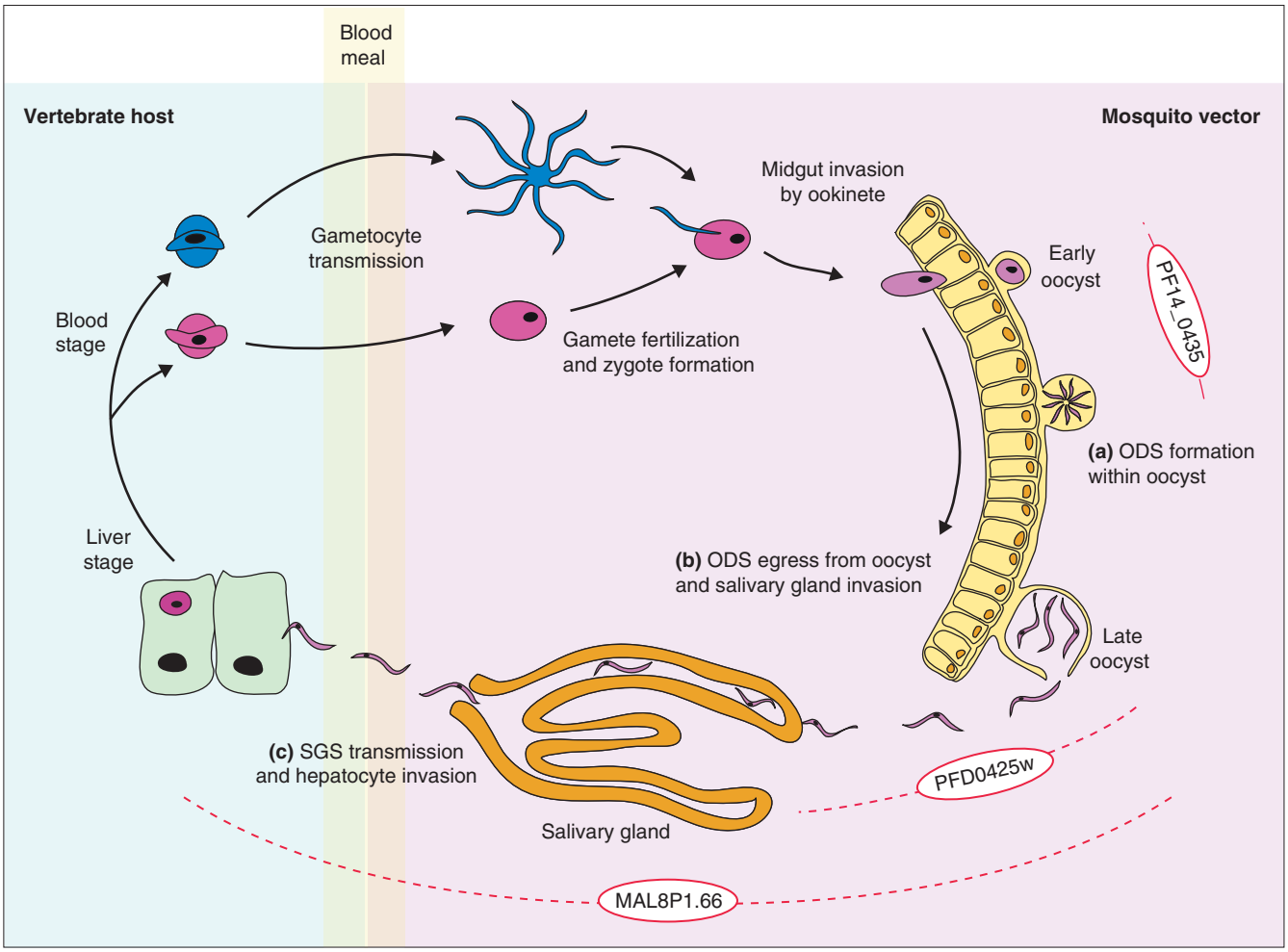

Figure 1
Pre-erythrocytic stages of the *Plasmodium* life cycle analyzed by comparative proteomics in the study of Lasonder *et al.* [2]. Mosquito stages are shown on a pale pink background; stages in the vertebrate host on a pale blue background. **(a)** Oocyst derived sporozoites (ODS) are generated within the oocyst embedded in the mosquito midgut. **(b)** ODS egress from the oocyst and invade salivary glands. **(c)** Salivary gland sporozoites (SGS) are transmitted to the vertebrate host and invade hepatocytes. Other stages included for reference include gametocyte maturation, gamete fertilization and zygote formation, which precede invasion of the mosquito midgut by the ookinete; and the liver and blood stages. The stage-specific proteins characterized by Lasonder *et al.* [2] (in red ovals) are depicted at the point of the cycle where they are predicted to function. Modified from Vickerman and Cox [26].

Identifying malaria control targets

While ODS are morphologically indistinguishable from the mature SGS, these forms carry out very different processes (Figure 1). ODS travel through the hemolymph of the mosquito to find, bind and traverse the salivary glands into the secretory cavity. In contrast, SGS released from the mosquito salivary gland into the vertebrate host have to navigate the skin and circulatory system of the host to reach the liver, and then traverse several liver cells before ultimately forming a parasitophorous vacuole membrane around itself within a hepatocyte. SGS and ODS should therefore have distinct protein repertoires to mediate their diverse functions. Proteins that are more abundant in ODS could have roles in sporozoite maturation within the oocyst, in egress, and in salivary gland recognition and invasion. Conversely, proteins that are more abundant in SGS could be involved in the establishment of infection in the human host, with possible roles in migration through skin, binding and traversing hepatic cells, and invading hepatocytes. In accordance with these different biological properties, SGS display a dramatically enhanced ability to invade hepatocytes and to establish infection in the vertebrate host when compared to ODS (reviewed in [12]), and the transcriptional profiles of ODS and SGS are different [13,14].

With this hypothesis in mind, Lasonder and colleagues isolated ODS and SGS by hand-dissection of infected mosquitoes and compared their proteomes by high-throughput nano-liquid chromatography tandem mass spectrometry [2]. Many of the 250 proteins identified

exclusively in mosquito stages (as determined by comparison with previously published data by the same group [15]), are annotated as hypothetical, meaning that their function cannot be inferred by sequence homology. Some of these are likely to carry out *Plasmodium*-specific functions relevant to malaria pathogenesis. While many proteins were detected in multiple mosquito stages, approximately 24% of the proteins in the SGS fraction and 15% of those in the ODS fraction were stage-specific. Importantly, the majority of proteins reported to be involved in sporozoite development and invasion of host cells were observed in the ODS and SGS proteomes. Proteins with multiple roles in sporozoite maturation and development (such as the inner membrane complex protein (IMC1), circumsporozoite surface protein (CSP) and thrombospondin-related anonymous protein (TRAP)) were identified in both stages. Conversely, the membrane antigen erythrocyte binding-like protein (MAEBL), which has been reported to function in attachment and invasion to the salivary gland, was detected at much higher levels in the ODS proteome, and proteins with functions in hepatocyte traversal or invasion (for example, sporozoite microneme proteins essential for cell traversal (SPECT1 and 2), cell-traversal protein for ookinetes and sporozoites (CelTOS), secreted protein with altered thrombospondin domain (SPATR), apical membrane antigen (AMA1), and the surface proteins P36p and P36) were exclusive or more abundant in the SGS proteome.

The study yielded three lists of proteins: those exclusive to the mosquito-stage proteome but shared between the different sporozoite forms, and those highly differentially enriched in either the SGS or the ODS proteome. On the basis of expression profiling, lack of functional annotation, presence of orthologs in the rodent species *P. berghei*, and bioinformatic predictions of parasite-specific protein secretion signals, eight proteins were selected for further analysis by targeted deletion of their orthologs in *P. berghei*. In total, six mutant lines were successfully generated (two genes could not be deleted, suggesting essential functions in blood-stage parasites). All mutant lines showed normal asexual development and gametocyte and ookinete production. Three of the six lines displayed no difference in the number of oocysts or sporozoites formed or in their ability to establish productive infections in mice when compared to wild-type parasites. This suggests that either the function of the corresponding proteins is not essential for mosquito- or liver-stage development, or alternatively that functional redundancy interfered with the identification of a phenotypic effect in the mutant lines.

The other three parasite lines (one mutant for an ODS-specific protein, and two with gene deletions for proteins shared by ODS and SGS) were unable to infect mice. Although all three mutant lines generated normal numbers of oocysts, they displayed distinct developmental defects. First, disruption of the *P. berghei* ortholog of PF14_0435 prevented sporozoite formation within otherwise morphologically normal oocysts. This early phenotype is reminiscent of sporogonic defects observed in loss-of-function mutants of the major sporozoite surface protein CSP (reviewed in [12]), and is consistent with expression of this protein by ODS. In contrast, deletion of the MAL8P1.66 ortholog resulted in a drastically reduced number of ODS that could nonetheless invade salivary glands and traverse hepatocytes. In spite of this, the resulting sporozoites were not infective to mice, possibly because the MAL8P1.66 orthologue is expressed by liver-stage parasites [16] and, therefore, may have an essential role in liver-stage development as well. Thus, the function of MAL8P1.66 might be required for a process that is important throughout the mosquito and liver stages.

Lastly, the parasite line mutated in the ortholog of *P. falciparum* PFD0425w generated normal numbers of ODS but displayed an egress defect that resulted in the accumulation of sporozoites within the oocyst and a low number of sporozoites in the hemolymph and the salivary glands. The authors point out that this phenotype is similar to that observed in mutants of the egress cysteine protease (ECP1) [17] and in parasite lines that carry mutations in the region II-plus domain of CSP [18]. Mosquitoes infected with this line were unable to cause infection in mice, although ODS collected mechanically from these mosquitoes and injected intravenously yielded typical levels of parasitemia in mice, leading Lasonder *et al.* [2] to speculate that this protein is not likely to have an essential role in later stages of the parasite life cycle.

P. falciparum PFD0425w, as well as its *P. yoelii* ortholog have been previously characterized as mosquito- and liver-stage specific proteins [19,20]. Importantly, volunteers immunized with irradiated sporozoites mount an antibody response against this protein, although antibody reactivity did not correlate with protection from malaria [21]. More recently, PFD0425w was identified as having a transient pattern of upregulation upon co-incubation of SGS with hepatocytes at 37°C, similar to that of other proteins that have been implicated in hepatocyte invasion [22]. Moreover, the presence of a signal peptide on PFD0425w, the immunofluorescent localization of the protein, and its role in sporozoite invasion and traversal of hepatocytes (suggested by antibody inhibition studies) indicate that this protein, like CSP, is displayed on the surface of SGS. Western blots suggest that PFD0425w might be proteolytically cleaved after exposure to host hepatocytes, as are AMA1 and TRAP [23]. From these results, Siau *et al.* [22] named the protein sporozoite invasion-associated protein-1 (SIAP-1), and proposed that its main function might be related to gliding, in apparent contradiction to the conclusions of Lasonder *et al.* [2].

Whether the different conclusions of Siau *et al.* [22] and Lasonder *et al.* [2] regarding the function of PFD0425w correspond to variations among species or reflect technical aspects of the assays was addressed by recent work from Matuschewski and his group (Engelmann *et al.* [24]). This new study confirms the oocyst egress defect resulting from the disruption of PFD0425w reported by Lasonder *et al.* [2]. However, using a green fluorescent protein-tagged version of the *P. berghei* ortholog of PFD0425w, Engelmann *et al.* [24] were able to detect low numbers of sporozoites in the hemocoel. Furthermore, in agreement with the interpretation of Siau *et al.* [22], their data support the notion that PFD0425w is involved in gliding locomotion within the mosquito, as the few sporozoites detected in the hemocoel displayed severely reduced motility, which may in turn result in their reduced ability to invade salivary glands. The authors note that both egress from oocysts and movement through the hemocoel are related to sporozoite locomotion, tying together the apparently disparate phenotypes assigned to this protein. The new data confirm that the protein is localized to the apical tip of both ODS and SGS, and, in agreement with previous reports, that the expression of the gene encoding *P. berghei* SIAP-1 is maximal in young oocysts and diminishes upon hepatocyte invasion [24]. Finally, when large numbers of ODS and SGS isolated from the *P. berghei* SIAP-1 mutant were injected into mice, a low percentage of the animals developed parasitemia, in concordance with the results obtained by Lasonder *et al.* with *P. yoelii* sporozoites obtained by mechanical disruption of oocysts [2].

Following the sporozoite trail

The work described above validates the usefulness of a proteome-reverse genetic approach for the characterization of proteins specific to a particular stage of the life cycle. Eight proteins were selected from the initial lists, and of the six that could be successfully disrupted, half displayed unique phenotypes, reflecting the participation of the proteins in different events during the sporogonic and liver stages of the parasite's life cycle. The list of sporozoite-specific proteins is bound to contain many other putative vaccine and drug targets. From the point of view of human disease, *P. falciparum*-specific proteins that lack rodent orthologs might be of special interest as they could encode functions needed exclusively for human pathogenesis. However, understanding the *in vivo* roles of these proteins will require the development of novel approaches.

These case studies in functional genomics show just how rapidly genome-scale technologies can change the face of translational research. The *P. falciparum* genome was published in 2002, and the subsequent six years have seen the discovery of a candidate for a vaccine aimed at preventing malaria infection during pregnancy as well as significant follow-up research validating this finding (reviewed in [25]). Recently, combined transcriptome and proteome analyses of the liver stages of *P. yoelii* revealed that, among others, proteins involved in the type II fatty-acid synthesis metabolic pathway are upregulated, and more importantly demonstrated that inhibitors of this pathway are effective drugs against liver-stage parasites [16]. Now, the sporozoite stages are following suit, pushing new vaccine and drug targets into the research pipeline.

References

1. World Health Organization: **World Malaria Report 2008.** Geneva: WHO Press; 2008.
2. Lasonder E, Janse CJ, van Gemert GJ, Mair GR, Vermunt AM, Douradinha BG, van Noort V, Huynen MA, Luty AJ, Kroeze H, Khan SM, Sauerwein RW, Waters AP, Mann M, Stunnenberg HG: **Proteomic profiling of Plasmodium sporozoite maturation identifies new proteins essential for parasite development and infectivity.** *PLoS Pathog* 2008, **4:**e1000195.
3. 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 MS, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DM, *et al.*: **Genome sequence of the human malaria parasite *Plasmodium falciparum*.** *Nature* 2002, **419:**498-511.
4. Carlton JM, Angiuoli SV, Suh BB, Kooij TW, Pertea M, Silva JC, Ermolaeva MD, Allen JE, Selengut JD, Koo HL, Peterson JD, Pop M, Kosack DS, Shumway MF, Bidwell SL, Shallom SJ, van Aken SE, Riedmuller SB, Feldblyum TV, Cho JK, Quackenbush J, Sedegah M, Shoaibi A, Cummings LM, Florens L, Yates JR, Raine JD, Sinden RE, Harris MA, Cunningham DA, *et al.*: **Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii* yoelii.** *Nature* 2002, **419:**512-519.
5. Hall N, Karras M, Raine JD, Carlton JM, Kooij TW, Berriman M, Florens L, Janssen CS, Pain A, Christophides GK, James K, Rutherford K, Harris B, Harris D, Churcher C, Quail MA, Ormond D, Doggett J, Trueman HE, Mendoza J, Bidwell SL, Rajandream MA, Carucci DJ, Yates JR 3rd, Kafatos FC, Janse CJ, Barrell B, Turner CM, Waters AP, Sinden RE: **A comprehensive survey of the Plasmodium life cycle by genomic, transcriptomic, and proteomic analyses.** *Science* 2005, **307:**82-86.
6. Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzi H, Caler E, Crabtree J, Angiuoli SV, Merino EF, Amedeo P, Cheng Q, Coulson RM, Crabb BS, Del Portillo HA, Essien K, Feldblyum TV, Fernandez-Becerra C, Gilson PR, Gueye AH, Guo X, Kang'a S, Kooij TW, Korsinczky M, Meyer EV, Nene V, Paulsen I, White O, Ralph SA, Ren Q, Sargeant TJ, *et al.*: **Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*.** *Nature* 2008, **455:**757-763.
7. Pain A, Böhme U, Berry AE, Mungall K, Finn RD, Jackson AP, Mourier T, Mistry J, Pasini EM, Aslett MA, Balasubrammaniam S, Borgwardt K, Brooks K, Carret C, Carver TJ, Cherevach I, Chillingworth T, Clark TG, Galinski MR, Hall N, Harper D, Harris D, Hauser H, Ivens A, Janssen CS, Keane T, Larke N, Lapp S, Marti M, Moule S, *et al.*: **The genome of the simian and human malaria parasite *Plasmodium knowlesi*.** *Nature* 2008, **455:**799-803.
8. Winzeler EA: **Malaria research in the post-genomic era.** *Nature* 2008, **455:**751-756.
9. Beier JC, Vanderberg JP: **Sporogenic development in the mosquito.** In *Malaria: Parasite Biology, Pathogenesis and Protection.* Edited by Sherman IW. Washington, DC: ASM Press; 1998: 48-62.
10. Clyde DF, Most H, McCarthy VC, Vanderberg JP: **Immunization of man against sporozite-induced falciparum malaria.** *Am J Med Sci* 1973, **266:**169-177.
11. Nussenzweig RS, Vanderberg J, Most H, Orton C: **Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*.** *Nature* 1967, **216:**160-162.
12. Kappe SH, Kaiser K, Matuschewski K: **The *Plasmodium* sporozoite journey: a rite of passage.** *Trends Parasitol* 2003, **19:**135-143.
13. Matuschewski K, Ross J, Brown SM, Kaiser K, Nussenzweig V, Kappe SH: **Infectivity-associated changes in the transcriptional repertoire of the malaria parasite sporozoite stage.** *J Biol Chem* 2002, **277:**41948-41953.

14. Mikolajczak SA, Silva-Rivera H, Peng X, Tarun AS, Camargo N, Jacobs-Lorena V, Daly TM, Bergman LW, de la Vega P, Williams J, Aly AS, Kappe SH: **Distinct malaria parasite sporozoites reveal transcriptional changes that cause differential tissue infection competence in the mosquito vector and mammalian host.** *Mol Cell Biol* 2008, 28:6196-6207.
15. Lasonder E, Ishihama Y, Andersen JS, Vermunt AM, Pain A, Sauerwein RW, Eling WM, Hall N, Waters AP, Stunnenberg HG, Mann M: **Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry.** *Nature* 2002, **419:**537-542.
16. 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 USA* 2008, **105:**305-310.
17. Aly AS, Matuschewski K: **A malarial cysteine protease is necessary for Plasmodium sporozoite egress from oocysts.** *J Exp Med* 2005, **202:**225-230.
18. Wang Q, Fujioka H, Nussenzweig V: **Exit of *Plasmodium* sporozoites from oocysts is an active process that involves the circumsporozoite protein.** *PLoS Pathog* 2005, **1:**e9.
19. Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD, Moch JK, Muster N, Sacci JB, Tabb DL, Witney AA, Wolters D, Wu Y, Gardner MJ, Holder AA, Sinden RE, Yates JR, Carucci DJ: **A proteomic view of the Plasmodium falciparum life cycle.** *Nature* 2002, **419:**520-526.
20. Kaiser K, Matuschewski K, Camargo N, Ross J, Kappe SH: **Differential transcriptome profiling identifies *Plasmodium* genes encoding pre-erythrocytic stage-specific proteins.** *Mol Microbiol* 2004, **51:**1221-1232.
21. Doolan DL, Southwood S, Freilich DA, Sidney J, Graber NL, Shatney L, Bebris L, Florens L, Dobano C, Witney AA, Appella E, Hoffman SL, Yates JR 3rd, Carucci DJ, Sette A: **Identification of P*lasmodium falciparum* antigens by antigenic analysis of genomic and proteomic data.** *Proc Natl Acad Sci USA* 2003, **100:**9952-9957.
22. Siau A, Silvie O, Franetich JF, Yalaoui S, Marinach C, Hannoun L, van Gemert GJ, Luty AJ, Bischoff E, David PH, Snounou G, Vaquero C, Froissard P, Mazier D: **Temperature shift and host cell contact up-regulate sporozoite expression of *Plasmodium falciparum* genes involved in hepatocyte infection.** *PLoS Pathog* 2008, **4:**e1000121.
23. Silvie O, Franetich JF, Charrin S, Mueller MS, Siau A, Bodescot M, Rubinstein E, Hannoun L, Charoenvit Y, Kocken CH, Thomas AW, Van Gemert GJ, Sauerwein RW, Blackman MJ, Anders RF, Pluschke G, Mazier D: **A role for apical membrane antigen 1 during invasion of hepatocytes by Plasmodium falciparum sporozoites.** *J Biol Chem* 2004, **279:**9490-9496.
24. Engelmann S, Silvie O, Matuschewski K: **Disruption of Plasmodium sporozoite transmission by depletion of the sporozoite invasion-associated protein-1.** *Eukaryot Cell* 2009, **8:**640-648.
25. Duffy PE: ***Plasmodium* in the placenta: parasites, parity, protection, prevention and possibly preeclampsia.** *Parasitology* 2007, **134:**1877-1881.
26. Vickerman K, Cox F: *The Protozoa.* London: Albemarle Street; 1972.