Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Rakhi Dhawan
Department of Community Medicine, Armed Forces Medical College, Sholapur Road, Pune-40

Ashwani Kumar
National Institute of Malaria Research, Field Station, Panaji, Goa 403001, India.

Gametocytogenesis in *Plasmodium falciparum* – A 'Parasite' view

Rakhi Dhawan, Ashwani Kumar

Abstract
The different morphological stages of *Plasmodium falciparum* show different physiological and biochemical characteristic. Lately there has been a renewed interest in the proteome of the parasite leading to considerable body of work towards identification of stage and sex specific proteins.
In 2002 a whole chromosome shotgun sequencing of *P falciparum* clone 3D7 was completed. The chromosomes of *P falciparum* show gene clustering with coregulated gene groups and proteins. The Parasitophorous Vacuole Membrane and Gametocyte Exported Proteins are seen to be over expressed during early phase of sexual differentiation. The gametocyte proteome is in general specific to differing functionalities of the sexes. All ribosomal proteins are highly expressed in females. The sensitivity of this stage to antimalarials attacking respiration like primaquine and artemisinin based drugs is pronounced. Research on on NF54 isolate of *P falciparum* has revealed that of the total of 1289 proteins found by nano LC MS/MS, 315 were solely in gametocytes. This is a large percentage of total and highlights the significance of dwelling deeper into the protein-protein interactions taking place at different stages of gametocyte development.

Keywords: gametocyte, proteome, genome, parasitophorous vacuole, signalling pathways

1. Introduction
Gametocytes in *Plasmodium falciparum* have five characteristic morphological stages (I to V) [1, 2]. Each stage undergoes distinct physiological as well as biochemical changes. The stage I gametocyte resembles asexual trophozoite whereas the stage IV and V show distinct sexual dimorphism. Mature female gametes are better prepared for extensive and rapid protein synthesis whereas its male counterpart has organelles favouring need for rapid cell division. Both contain a single mitochondria which is a large branching structure and can be identified throughout all gametocyte stages. It has an established relation with apicoplast [3-7]. RNA synthesis terminates in stage IV-V. Microgametes resume the arrested G0 phase of cell cycle only once it reaches the midgut of mosquito vector and gets activated for exflagellation [8].

2. Review
2.1 The Parasite Genome
A whole chromosome shotgun sequencing of *P falciparum* clone 3D7 was completed in 2002 [12]. Gardner M J *et al.* reported 14 chromosomes encoding 5300 genes with most (A+T) rich genome sequenced so far. The nuclear genome is 23Kb while the mitochondrial genome is only 6 Kb without any t- RNA encoding potential which are imported in it [10, 11]. Mitochondrial enzymes, proteins and ribosomes are seemingly more abundant in female gamete consonant with their greater role in protein synthesis [4]. There is evidence that mitochondria and apicoplast are maternally inherited [13, 14]. The apicoplast harboured by members of phylum Apicomplexa is considered necessary for survival of the parasite. It has a 35 Kb genome encoding 30 proteins [15]. An additional 550 nuclear encoded proteins are targeted into the organelle. Half of these are yet of unknown functionality [16]. It has a role in anabolic synthesis of fatty acids, heme and isoprenoids [17-21]. The apicoplast genome has t-RNA encoding potential [22].
The parasite has single 18s-5.8s-28s r-RNA on several chromosomes with a total of seven encoding loci. S-type r-RNA encoding genes are expressed in mosquito vector whereas a type are expressed primarily inside human host [9]. Gametocytes do not undergo genome replication. Translational repression of m-RNA encoding several important proteins is known to occur till the gametocyte reaches mosquito life cycle stage [23, 24]. This mechanism allows

Correspondence
Ashwani Kumar
National Institute of Malaria Research, Field Station, Panaji, Goa 403001, India.

piling of m-RNA awaiting favourable environmental conditions where the requirement of excess proteins is anticipated [25].

2.2 The Subtelomeric Regions

The chromosome show size polymorphism which is said to be a function of meiotic recombination. The subtelomeric regions are highly conserved and seem to play a major role in antigenic variation [26] they contain blocks of repetitive sequences called TARE (Telomere associated repetitive element) which are six in number [27, 28]. The chromosomes of *P falciparum* show gene clustering. Analysis of these co regulated gene groups with co expressed proteins can assist in predicting protein function based on their association [29]. Karine G. Le Roch *et al*. used a high density oligonucleotide array and generated a profile of all stages of the malaria parasite and provided functional information of the predicted genes using probes from coding as well as non coding sequence. They proposed that genes with similar function have similar expression profile and are co located on the chromosome [30].

2.3 The Gene Families

There are three prominent gene families clustered around telomeres and are responsible for immune evasion. These are var, rif and stevor. The protein coded by them are called Pf EMP1 (*P falciparum* Erythrocyte Membrane Protein 1), rifin (repetitive interspersed family) and stevor (subtelomeric variable open reading frame) respectively. PfEPM1 mediate cytoadherence causing cell sequestration and severe disease. The family of Pf EMP1proteins is found only in early gametocyte stage and is encoded by nearly 60 var genes [31]. The knobs on the surface of infected erythrocytes harbour these proteins which bind to host receptors in early gametocyte stage. Cytoadherence in later stages appear to be a host receptor response [32-35]. Var gene family is mainly responsible for pathogenesis, chronic infection, transmission and induction of protective immunity [36]. Rifins cause antigenic variation on infected red cell surface. Stevor proteins are encoded by 40-50 stevor genes. They are present on Maurer's cleft and peak at 28 hr post invasion. They are expressed in stage- I but transported to infected erythrocytes in stage-III [37, 38].

2.4 The Parasitophorous Vacuole

A Parasitophorous Vacuole (PV) is formed inside the parasite initially by liver cell plasma membrane when infected by a sporozoite and again from RBC plasma membrane during invasion of RBC by merozoites. The basic lipid structure of this parasitophorous vacuole membrane (PVM) is initially derived from the host cells and later modified by parasite itself. The parasite eventually leaves this PVM inside the mosquito host after rounding up and emergence from RBC [39-41].

Pfs16 has been studied vastly as a specific indicator protein for gametocytogenesis [42, 43]. It is present on the PVM and is small (16KDa) and expressed throughout all stages of gametocytes. Recently Saliha Eksi *et al*. reported the amino acid domain required for targeting *P falciparum* PVM employing Pfs16-GFP (Green Fluorescent Protein) parasite lines assayed in vivo by epifluorescence microscopy [42].

Among all the proteins associated with PVM, EXP-1(PF11-0224) and ETRAMP family are the only ones which have been well characterised so far [44-46].

2.5 Gametocyte Exported Proteins

In another recent research study Silvestrini *et al*. obtained a highly purified transgenic line of early gametocytes and through proteomics approach detected 1427 proteins at early gametocyte stages and another 2031 at mature stages. Out of these 1090 were novel. They observed that proteins exported in erythrocyte cytoplasm and responsible for its remodelling during gametocyte development are the most over represented proteins of all. They are named PfGEXPs (*P falciparum* Gametocyte Exported Proteins). The export occurs during early sexual differentiation [47]. 40% of these 26 putatively exported proteins belong to PHIST gene family. They demonstrated six over represented gene sets in early gametocytes (stage I & II). The most significant of these being the ones encoding *Plasmodium* exported proteins. The other important ones were for host cell remodelling and chaperonins [48].

In experiments conducted in vitro, gametocyte production and cytoadherence have been shown to be associated with subtelomeric region of chromosome 9 containing 15 genes annotated so far [49, 50]. Pfgig is a noted member of this group proposed to be responsible for regulating the commitment to sexual differentiation. Its disruption decreases gametocyte production [51].

2.6 Proteome of Gametocytes

Khan *et al*. report a sex specific gametocyte proteome of *P berghei* with only 69 out of a total of 475 proteins being shared by male and female gametes in their study [52]. Florence *et al*. [29] undertook functional profiling of 2415 proteins covering four major stages of parasite; sporozoite, merozoite, trophozoite and gametocytes by Mud PIT (Multidimensional Protein Identification Technology) and sorted proteins in functional classes as per MIPS catalogue.(Munich Information center for Protein Sequences) [53]. As per them the gametocyte proteome is in general specific to differing functionalities of the sexes. The females are loaded with ribosomes and Endoplasmic Reticulum for translation initiation and contain m-RNA encoding surface antigens of gamete, zygote and ookinete stages. All ribosomal proteins are highly expressed in females. The sensitivity of this stage to antimalarials attacking respiration like primaquine and artemisinin based drugs is pronounced due to developed mitochondrial functionality [54]. Lasonder *et al*. worked on NF54 isolate of *P falciparum* and found that of the total of 1289 proteins revealed by nano LC MS/MS, 315 were solely in gametocytes. As per there finding m RNA from Pfg 377, gene11-1 and the protein Pf14_0039 were exclusive to gametocytes and gametes [55].

2.7 Signal Transduction Pathways in Gametocytes

The major signalling pathways described so far in *Plasmodium falciparum* include calcium, c-AMP, c-GMP and PDE (Phosphodiesterase) dependent pathways. In addition to this CDPK family (Calcium Dependent Phospho Kinase) is a prominent calss of downstream effector of Ca^{2+} mediated signal transduction. Certain receptor families have established themselves as involved in physiological responses to stimulus.

These include GPCR (G-Protein Coupled Receptor) and serpentine receptors [56, 57]

Role of indole as an important signalling molecule [58] and melatonin treatment causing ca2+ release with subsequent erythrocyte rupture, Hb and cytoskeleton degradation [59] are still under investigation.

c-AMP and c-GMP are important second messengers synthesised by PfAC (Adenylyl Cyclase) and PfGC (Guanylyl Cyclase). The genome of *P falciparum* encodes two PfACs, PfACα AND PfACβ [58]. The role of c-AMP pathway in gametocyte induction was demonstrated in vitro by treating culture with its agonists and showing increased gametocytogenesis. It is suggested that c-AMP may have a role in sexual differentiation of malarial parasite [59-64]. C-AMP further activates Protein Kinases (PKA). There are two c-AMP dependent PKAs and one c-GMP dependent PKG. c-AMP and c-GMP are degraded by four PDEs [65, 66].

Calcium based signalling has been reported during erythrocytic schizogony, gametogenesis and ookinete motility [67, 68]. On the other hand, c-GMP activates PKG and the calcium release. This is found to be a crucial step in xanthurenic acid induced gametogenesis in Pf [69] ookinete gliding activity [70] and sporozoite motility [71-73]. *P falciparum* has two guanylyl cyclases, PfGCα and PfGCβ. PfGCα is essential for both sexual and asexual stages. c-GMP was earlier demonstrated to be having a role in exflagellation. Disruption of PfPDE gene has been shown to decrease c-GMP levels and affect gametogenesis [74]. This appears to be an interesting target for further studies on sexual development of *P falciparum.*

More work is in progress on role of InsP3 produced by gametocytes in increase in calcium levels during exflagellation [75, 76] and also on the PfCDPK genes which are expressed largely during sexual phase [77] amongst its several members, CDPK4 is a key enzyme in male gamete formation and stimulation of S-phase of cell cycle. The infectivity of ookinete which are formed from CDPK4 deficient macrogametes is also reduced [78]. CDPK3 has exclusive expression in ookinetes and its deficiency in *P berghei* lines has shown reduced transmission efficiency by reduced generation of oocyst in midgut [79, 80].

3. Conclusion

The proteomics of gametocytes holds promising results opportunities for deeper understanding of the sexual stage of the parasite. The subtelomeric regions are highly conserved and seem to play a major role in antigenic variation. Study of co regulated gene groups with co expressed protein regions can predict functionality of proteins based on the location.

The multigene families clustered around telomere seem to be having a supporting role in early gametocyte stage cytoadherence and sequestration. The major role players that can be focussed as targets for prevention of severe disease still need scientific evidence.

The role of the highly active protein export process in early gametocytogenesis need further evidence based studies. It may bring to light physiological phenomena responsible for sequestration and severity of disease in sexual stage. The role of PV in this process is worth further elucidation. It is also noteworthy that not many research work have deliberated on sex specific proteogenomic analysis of sexual development.

The parasite metabolic pathways are stage specific with TCA cycle and Oxidative phosphorylation enzymatic peptides being most prominent in gametocytes. This existing information brings out a need to perform stage specific proteogenomic analysis and a detailed study of protein-protein interactions at every stage.

List of Abbreviations

TARE	Telomere associated repetitive element
EMP	Erythrocyte Membrane Protein
rifin	Repetitive interspersed family
stevor	Subtelomeric variable open reading frame
PV	Parasitophorous Vacuole
PVM	Parasitophorous Vacuole Membrane
GFP	Green Fluorescent Protein
PfGEXPs	*P falciparum* Gametocyte Exported Proteins
Mud PIT	Multidimensional Protein Identification Technology
MIPS	Munich Information center for Protein Sequences
CDPK	Calcium Dependent Phospho Kinase
GPCR	G-Protein Coupled Receptor
AC	Adenylyl Cyclase
GC	Guanylyl Cyclase
PKA	Activates Protein Kinase

Competing interests

Author Contribution
AK conceived the topic, guided in review of literature, edited the manuscript, provided critical inputs and revised the manuscript. RD reviewed the literature and prepared manuscript. Both authors read and approved the final manuscript.

Author Information
RD is MD in Community Medicine from Forces Medical College, Pune, India and is faculty at the Dept of Community Medicine, AFMC, Pune. She is pursuing PhD in Medical Entomology at National Institute of Malaria Research, New Delhi. AK is Deputy Director and Officer-In-Charge, National Institute of Malaria Research (ICMR), DHR, Govt. of India Goa. He is also member of Vector Control Advisory Group (NTD), Evidence Review Group (MPAC, GMP), WHO, Geneva and Team Leader of Epidemiology (NIH sponsored - Intl. Center of Excellence for Malaria Research) in Malaria Evolution in South Asia Project.

4. References
1. Carter R, Miller LH. Evidence for environmental modulation of gametocytogenesis in *Plasmodium falciparum* in continuous culture. Bulletin of the World Health Organization 1979; 57(1):37-52.
2. Zhenyu Liu, Jun Miao, Liwang Cui. Gametocytogenesis in malaria parasite: commitment, development and regulation, Future Microbiol 2011; 6(11):1351-1369.
3. Sinden RE. Sexual development of malarial parasites. Adv. Parasitol 1983; 22:153-216.

4. Sinden RE. Gametocytogenesis of *Plasmodium falciparum in vitro*: an electron microscopic study. Parasitology 1982; 84(1):1-11.

5. Sinden RE, Butcher GA, Billker O, Fleck SL. Regulation of infectivity of Plasmodium to the mosquito vector. Adv. Parasitol 1996; 38:53-117.

6. Okamoto N, Spurck TP, Goodman CD, McFadden GI. Apicoplast and mitochondrion in gametocytogenesis of *Plasmodium falciparum*. Eukaryot. Cell 2009; 8(1):128-132.

7. Van Dooren GG, Stimmler LM, McFadden GI. Metabolic maps and functions of the Plasmodium mitochondrion. FEMS Microbiol. Rev 2006; 30(4):596-630.

8. Talman AM, Domarle O, McKenzie FE, Ariey F, Robert V. Gametocytogenesis: the puberty of *Plasmodium falciparum*. Malaria 2004; 3:24.

9. Gardner MJ, Hall N, Fung E. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 2002; 419(6906):498-511.

10. Vaidya AB, Akella R, Suplick K. Sequences similar to genes for two mitochondrial proteins and portions of ribosomal RNA in tandemly arrayed 6-kilobase-pair DNA of a malaria parasite. Mol. Biochem. Parasitol 1989; 35:97-107.

11. Vaidya AB, Lashgari MS, Pologe LG. Morrisey J. Structural features of Plasmodium cytochrome b that may underlie susceptibility to 8-aminoquinolines and hydroxynaphthoquinones. Mol. Biochem. Parasitol 1993; 58:33-42.

12. Carter R, Ranford-Cartwright L, Alano P. The culture and preparation of gametocytes of *Plasmodium falciparum* for immunochemical, molecular, and mosquito infectivity studies. Methods Mol Biol 1993; 21:67-88.

13. Creasey AM, Ranford-Cartwright LC, Moore DJ. Uniparental inheritance of the mitochondrial gene cytochrome b in *Plasmodium falciparum*. Curr Genet 1993; 23:360-364.

14. Vaidya AB, Morrisey J, Plowe CV, Kaslow DC, Wellems TE. Unidirectional dominance of cytoplasmic inheritance in two genetic crosses of *Plasmodium falciparum*. Mol Cell Biol 1993; 13:7349-7357.

15. Wilson RJM. Complete gene map of the plastid-like DNA of the malaria parasite *Plasmodium falciparum*. J Mol Biol. 1996; 261:155-172.

16. Ralph SA, D'Ombrain MC, McFadden GI. The apicoplast as an antimalarial drug target. Drug Resist. Update. 2001; 4:145-151.

17. Waller RF. Nuclear-encoded proteins target to the plastid in Toxoplasma gondii and *Plasmodium falciparum*. Proc. Natl Acad. Sci. USA 1998; 95:12352-12357.

18. Surolia N, Surolia A. Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase of *Plasmodium falciparum*. Nature Med 2001; 7:167-173.

19. Sato S, Wilson RJ. The genome of *Plasmodium falciparum* encodes an active deltaaminolevulinic acid dehydratase. Curr. Genet 2002; 40:391-398.

20. Van Dooren GG, Su V, D'Ombrain MC, McFadden GI. Processing of an apicoplast leader sequence in *Plasmodium falciparum* and the identification of a putative leader cleavage enzyme. J Biol Chem. 2002; 277:23612-23619.

21. Gardner MJ. Chromosome 2 sequence of the human malaria parasite *Plasmodium falciparum*. Science 1998; 282:1126-1132.

22. Wilson RJM. Complete gene map of the plastid-like DNA of the malaria parasite *Plasmodium falciparum*. J Mol Biol. 1996; 261:155-172.

23. Hall N, Karras M, Raine JD. A comprehensive survey of the Plasmodium life cycle by genomic, transcriptomic, and proteomic analyses. Science. 2005; 307:82-86. [PubMed]

24. Le Roch KG, Johnson JR, Florens L. Global analysis of transcript and protein levels across the *Plasmodium falciparum* life cycle. Genome Res. 2004; 14:2308-2318.

25. Bhattacharyya MK, Kumar N. Effect of xanthurenic acid on infectivity of *Plasmodium falciparum* to Anopheles stephensi. International journal for parasitology. 2001; 31(10):1129-1133.

26. Hinterberg K, Mattei D, Wellems TE, Scherf A. Interchromosomal exchange of a large subtelomeric segment in a *Plasmodium falciparum* cross. EMBO J. 1994; 13:4174-4180.

27. Figueiredo LM, Freitas-Junior LH, Bottius E, Olivo-Marin JC, Scherf A. A central role for *Plasmodium falciparum* subtelomeric regions in spatial positioning and telomere length regulation. EMBO J. 2002; 21:815-824.

28. Scherf A, Figueiredo LM, Freitas-Junior LH. Plasmodium telomeres: a pathogen's perspective. Curr. Opin. Microbiol. 2001; 4:409-414.

29. Florens L, Washburn MP, Raine JD. A proteomic view of the *Plasmodium falciparum* life cycle. Nature 2002; 419(6906):520-526.

30. Karine G, Le Roch, Yingyao Zhou, Peter Blair L. Discovery of Gene Function by Expression Profiling of the Malaria parasite life cycle. Science 2003; 301:1503.

31. Kyes SA, Kraemer SM, Smith JD. Antigenic variation in *Plasmodium falciparum*: gene organization and regulation of the var multigene family. Eukaryot. Cell 2007; 6(9):1511-1520.

32. Rogers NJ, Hall BS, Obiero J, Targett GA, Sutherland CJ. A model for sequestration of the transmission stages of *Plasmodium falciparum*: adhesion of gametocyte-infected erythrocytes to human bone marrow cells. Infect. Immun 2000; 68(6):3455-3462.

33. Day KP, Hayward RE, Smith D, Culvenor JG. CD36-dependent adhesion and knob expression of the transmission stages of *Plasmodium falciparum* is stage specific. Mol. Biochem. Parasitol 1998; 93(2):167-177.

34. Hayward RE, Tiwari B, Piper KP, Baruch DI, Day KP. Virulence and transmission success of the malarial parasite *Plasmodium falciparum*. Proc. Natl Acad. Sci. USA 1999; 96(8):4563-4568.

35. Smith TG, Serghides L, Patel SN, Febbraio M, Silverstein RL, Kain KC. CD36-mediated nonopsonic phagocytosis of erythrocytes infected with stage I and IIA gametocytes of *Plasmodium falciparum*. Infect. Immun. 2003; 71(1):393-400.

36. Kyes S, Horrocks P, Newbold C. Antigenic variation at the infected red cell surface in malaria. Annu. Rev. Microbiol 2001; 55:673-707.

37. Sharp S, Lavstsen T, Fivelman QL. Programmed transcription of the var gene family, but not of stevor, in

Plasmodium falciparum gametocytes. Eukaryot. Cell 2006; 5(8):1206-1214.

38. McRobert L, Preiser P, Sharp S. Distinct trafficking and localization of STEVOR proteins in three stages of the *Plasmodium falciparum* life cycle. Infect. Immun 2004; 72(11):65976602.

39. Cowman AF, Crabb BS. Invasion of red blood cells by malaria parasites. Cell 2006; 124:755-766.

40. Cesbron-Delauw MF, Gendrin C, Travier L, Ruffiot P, Mercier C. Apicomplexa in mammalian cells: trafficking to the parasitophorous vacuole. Traffic 2008; 9:657-664.

41. Quakyi IA. Movement of a falciparum malaria protein through the erythrocyte cytoplasm to the erythrocyte membrane is associated with lysis of the erythrocyte and release of gametes. Infect. Immun. 1989; 57:833-839.

42. Saliha Eksi, Kim C. Williamson. Protein Targeting to the Parasitophorous Vacuole Membrane of *Plasmodium falciparum*. Eukaryotic Cell 2011; 10(6):744-752.

43. Silvestrini F. Genome-wide identification of genes upregulated at the onset of gametocytogenesis in *Plasmodium falciparum*. Mol. Biochem. Parasitol. 2005; 143:100-110.

44. Simmons D, Woollett G, Bergin-Cartwright M, Kay D, Scaife J. A malaria protein exported into a new compartment within the host erythrocyte. EMBO J 1987; 6:485-491.

45. Spielmann T, Fergusen DJ, Beck HP. etramps, a new *Plasmodium falciparum* gene family coding for developmentally regulated and highly charged membrane proteins located at the parasite-host cell interface. Mol. Biol. Cell 2003; 14:1529-1544.

46. Stenzel DJ, Kara UA. Sorting of malarial antigens into vesicular compartments within the host cell cytoplasm as demonstrated by Immuno electron microscopy. Eur. J Cell Biol. 1989; 49:311-318.

47. Silvestrini F, Lasonder E, Olivieri A. Protein export marks the early phase of gametocytogenesis of the human malaria parasite *Plasmodium falciparum*. Mol. Cell. Proteom. 2010; 9(7):1437-1448.

48. Sargeant TJ, Marti M, Caler E. Lineage-specific expansion of proteins exported to erythrocytes in malaria parasites. Genome. Biol. 2006; 7(2):R12.

49. Alano P, Roca L, Smith D, Read D, Carter R, Day K. *Plasmodium falciparum*: parasites defective in early stages of gametocytogenesis. Exp. Parasitol. 1995; 81(2):227-235.

50. Day KP, Karamalis F, Thompson J. Genes necessary for expression of a virulence determinant and for transmission of *Plasmodium falciparum* are located on a 0.3-megabase region of chromosome 9. Proc. Natl Acad. Sci. USA 1993; 90(17):8292-8296.

51. Gardiner DL, Dixon MW, Spielmann T. Implication of a *Plasmodium falciparum* gene in the switch between asexual reproduction and gametocytogenesis. Mol. Biochem. Parasitol. 2005; 140(2):153-160.

52. Khan SM, Franke-Fayard B, Mair GR. Proteome analysis of separated male and female gametocytes reveals novel sex-specific Plasmodium biology. Cell 2005; 121(5):675-687.

53. Mewes HW. MIPS: a database for genomes and protein sequences. Nucleic Acids Res 2002; 30:31-34.

54. Sinden RE, Butcher GA, Billker O, Fleck SL. Regulation of infectivity of Plasmodium to the mosquito vector. Adv. Parasitol. 1996; 38:53-117.

55. Lasonder E, Ishihama Y, Andersen JS. Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrome. Nature. 2002; 3, 419(6906):537-42.

56. Fernanda Koyama C, Debopam Chakrabarti, Célia Garcia RS. Molecular machinery of signal transduction and cell cycle regulation in Plasmodium. Mol Biochem Parasitol. 2009; 165(1):1-7.

57. Madeira L, Galante PA, Budu A, Azevedo MF, Malnic B, Garcia CR. Genome-wide detection of serpentine receptor-like proteins in malaria parasites. PLoS ONE; 2008; 3(3):e1889.

58. Chant EL, Summers DK. Indole signalling contributes to the stable maintenance of Escherichia coli multicopy plasmids. Mol Microbiol 2007; 63(1):35-43.

59. Farias SL, Gazarini ML, Melo RL. Cysteine-protease activity elicited by Ca2+ stimulus in Plasmodium. Mol Biochem Parasitol 2005; 141(1):71-79.

60. Muhia DK, Swales CA, Eckstein-Ludwig U. Multiple splice variants encode a novel adenylyl cyclase of possible plastid origin expressed in the sexual stage of the malaria parasite *Plasmodium falciparum*. J Biol Chem. 2003; 278(24):22014-22022.

61. Kaushal DC, Carter R, Miller LH, Krishna G. Gametocytogenesis by malaria parasites in continuous culture. Nature 1980; 286(5772):490-492.

62. Trager W, Gill GS. *Plasmodium falciparum* gametocyte formation in vitro: its stimulation by phorbol diesters and by 8-bromo cyclic adenosine monophosphate. J Protozool. 1989; 36(5):451-454.

63. Brockelman CR. Conditions favoring gametocytogenesis in the continuous culture of *Plasmodium falciparum*. J Protozool. 1982; 29(3):454-458.

64. Dyer M, Day K. Expression of *Plasmodium falciparum* trimeric G proteins and their involvement in switching to sexual development. Mol Biochem Parasitol; 2000; 110:437-448. [PubMed: 11071298]

65. Baker DA. Adenylyl and guanylyl cyclases from the malaria parasite *Plasmodium falciparum*. IUBMB Life 2004; 56(9):535-540.

66. Baker DA, Kelly JM. Purine nucleotide cyclases in the malaria parasite. Trends Parasitol. 2004; 20(5):227-232.

67. Billker O, Dechamps S, Tewari R, Wenig G, Franke-Fayard B, Brinkmann V. Calcium and a calcium dependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. Cell; 2004; 117(4):503-514. [PubMed: 15137943]

68. Caldas ML, Wasserman M. Cytochemical localisation of calcium ATPase activity during the erythrocytic cell cycle of *Plasmodium falciparum*. Int J Parasitol. 2001; 31(8):776-782. [PubMed:11403768]

69. McRobert L, Taylor CJ, Deng W. Gametogenesis in malaria parasites is mediated by the cGMPdependent protein kinase. PLoS Biol 2008; 6(6):e139. [PubMed: 18532880]

70. Ishino T, Orito Y, Chinzei Y, Yuda M. A calcium-dependent protein kinase regulates Plasmodium ookinete access to the midgut epithelial cell. Mol. Microbiol 2006; 59(4):1175-1184.

71. Coppi A, Tewari R, Bishop JR. Heparan sulfate proteoglycans provide a signal to Plasmodium sporozoites to stop migrating and productively invade host cells. Cell Host Microbe 2007; 2(5):316-327.

72. Doi Y, Shinzawa N, Fukumoto S, Okano H, Kanuka H. Calcium signal regulates temperature-dependent transformation of sporozoites in malaria parasite development. Exp. Parasitol. 2011; 128(2):176-180.

73. Kebaier C, Vanderberg JP. Initiation of Plasmodium sporozoite motility by albumin is associated with induction of intracellular signalling. Int. J Parasitol. 2011; 40(1):25-33.

74. Taylor CJ, McRobert L, Baker DA. Disruption of a *Plasmodium falciparum* cyclic nucleotide phosphodiesterase gene causes aberrant gametogenesis. Mol Microbiol. 2008; 69(1):110-118. [PubMed: 18452584]

75. Martin SK, Jett M, Schneider I. Correlation of phosphoinositide hydrolysis with exflagellation in the malaria microgametocyte. J Parasitol. 1994; 80(3):371-378. [PubMed: 8195938]

76. Passos A, Garcia C. Inositol 1,4,5-trisphosphate induced Ca2+ release from chloroquine-sensitive and -insensitive intracellular stores in the intraerythrocytic stage of the malaria parasite P. chabaudi. Biochem Biophys Res Commun 1998; 245:155-160. [PubMed: 9535800]

77. Ward P, Equinet L, Packer J, Doerig C. Protein kinases of the human malaria parasite *Plasmodium falciparum*: the kinome of a divergent eukaryote. BMC Genome 2004; 5(1):79.

78. Billker O, Dechamps S, Tewari R, Wenig G, Franke-Fayard B, Brinkmann V. Calcium and a calciumdependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. Cell 2004; 117(4):503-514. [PubMed: 15137943]

79. Ishino T, Orito Y, Chinzei Y, Yuda M. A calcium-dependent protein kinase regulates Plasmodium ookinete access to the midgut epithelial cell. Mol Microbiol 2006; 59(4):1175-1184. [PubMed: 16430692]

80. Siden-Kiamos I, Ecker A, Nyback S, Louis C, Sinden RE, Billker O. Plasmodium berghei calcium dependent protein kinase 3 is required for ookinete gliding motility and mosquito midgut invasion. Mol Microbiol 2006; 60(6):1355-1363. [PubMed: 16796674]