Gametocytogenesis in *Plasmodium falciparum* – A ‘Parasite’ view

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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...
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 stever. The protein coded by them are called PfEMP1 ( *P. falciparum* Erythrocyte Membrane Protein 1), rifin (repetitive interspersed family) and stever (subtelomeric variable open reading frame) respectively. PfEMP1 mediate cytoadherence causing cell sequestration and severe disease. The family of PfEMP1proteins 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 [30]. Rifins cause antigenic variation on infected red cell surface. Stevor proteins are encoded by 40-50 stever 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 Salih 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 PfGEXP's ( *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 signallong 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 cals of downstream effector of Ca2+ 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 melanotin 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 Plasmodium 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 ookinetocyte 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] ookinetocyte 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 PIPDE 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 ookinetocyte which are formed from CDPK4 deficient macrogametes is also reduced [78]. CDPK3 has exclusive expression in ookinetocytes 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
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

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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.

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