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Chapter

Molecular Dynamics of Mosquito-*Plasmodium vivax* Interaction: A Smart Strategy of Parasitism

Charu Chauhan, Sanjay Tevatiya, Seena Kumari, Punita Sharma, Jyoti Rani and Rajnikant Dixit

Abstract

Parallel to *Plasmodium falciparum*, *P. vivax* is a fast emerging challenge to control malaria in South-East Asia regions. Owing to unique biological differences such as the preference for invading reticulocytes, early maturation of sexual stages during the infection, the formation of hypnozoites, unavailability of *in-vitro* culture, the molecular relation of *P. vivax* development inside the mosquito host is poorly known. In this chapter, we briefly provide a basic overview of Mosquito-*Plasmodium* interaction and update current knowledge of tissue-specific viz. midgut, hemocyte, and salivary glands- molecular dynamics of *Plasmodium vivax* interaction during its developmental transformation inside the mosquito host, in specific.

Keywords: malaria, mosquito, *Anopheles*, *Plasmodium vivax*, host–parasite interaction

1. Introduction

The apicomplexan parasite *Plasmodium*, which is accountable for malaria, has a complex life cycle that includes both vertebrate hosts and invertebrate mosquitoes. Adaptation to blood-feeding in mosquitoes has made it inadvertently a carrier of various diseases. A blood meal is indispensable for adult female mosquitoes to nourish its egg, and maintain the gonotrophic cycle. But during blood feeding, ingestion of *Plasmodium* gametocyte from an infected person's blood results in the onset of 18-20 days long sporogonic cycle that culminates in the production of infectious sporozoites in the mosquito host [1]. These infectious sporozoites are then delivered into the human body through salivary discharge, which initiates the intricate stages of the asexual process causing malaria. In humans, malaria is caused by five *Plasmodium* species i.e.*, Plasmodium falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi* [2].

P. falciparum and *P. vivax*, vectored by the adult female *Anopheline* mosquitoes, are two principal parasites of human malaria [3]. Of the five *Plasmodium* species that cause human malaria, *Plasmodium vivax* is the most geographically widespread [4]. The parasite could survive quiescent for extended periods when circumstances

are not conducive to its ongoing transmission [5]. According to the current report by WHO, in the year 2019 around 75% of malaria cases were caused by *P. vivax* in the WHO Region of the Americas. An approximated 52% of the global burden of *P. vivax* emerged from the WHO South-East Asia Region, among which 47% were contributed from India [6].

P. vivax is considered as a less fatal parasite, but the recent emergence of more *P. vivax* infected cases in *P. falciparum* endemic areas, and increased mortality, morbidity rates are drawing our attention to this least studied parasite. It is more difficult to monitor and eradicate the *P. vivax* than *P. falciparum*, because of limited information, and associated biological complexities of its development in the mosquito as well as the human host [7, 8].

P. vivax normally circulates at low peripheral parasite densities, but still, they are transmissible by the mosquito vectors, and hence presents major challenges for the diagnosis of infected peoples. *P. vivax* has adapted to live with varying *Anopheles* vectors in different ecological conditions. Unlike other *Plasmodium* species, *P. vivax* has the potential to form dormant hypnozoites inside the host liver, and these liver-stage parasites are accountable for malaria relapses for weeks or months after initial infection [5]. Lastly, the lack of long-term *in-vitro* culture further restricts our understanding of the biological consequences of *P. vivax* development and transmission [9]. Nevertheless, for the last two decades, the integration and utilization of high-throughput molecular technologies such as genomics, RNA-Seq/transcriptomics, proteomics, have been valuable to decode and trace the genetic variation and diversity in the *P. vivax* population collected from different geographical origins [10, 11]. Efforts are continuing to uncover molecular and functional correlation of tissue/stage-specific *P. vivax* biology in the vertebrate host, identify genetic signatures to develop new diagnosis tools, anti-*P. vivax* drugs, or vaccine development. However, the biological complexity of the *P. vivax* development cycle in the mosquito vector-host is too limited, and therefore in this article, we highlight the current progress made so far in the understanding of the Mosquito-*P. vivax* interaction biology.

2. A general overview of the sporogonic cycle in mosquito host

The transmission of the parasite from human host to mosquito transpire when a female mosquito acquires gametocyte containing blood meal from the infected vertebrate host. When the parasite enters the midgut lumen it faces the dynamically changing environment, where both male and female gametocytes get differentiated into male and female gametes [12–15]. Ingested gametocytes also encounter proteolytic enzymes released by midgut epithelium in the midgut environment to digest the blood meal, which may have an agonistic or antagonistic effect on parasite growth. Fertilization of male and female gametes results in zygote formation, which rapidly transforms into motile ookinetes [16]. After exiting from the blood bolus, ookinete traverses the midgut epithelium either through intracellular or intercellular route and then rests beneath the epithelial cell at basal lamina. Later ookinetes transform into replicative oocyst stage which undergoes an umpteen round of nuclear division to produce thousands of sporozoites within a time period of one to two weeks. Once in the hemolymph circulation, the free circulatory sporozoites (*fcSPZ*) target to invade salivary glands, but most of them are rapidly cleared off by hemocytes, the immune blood cells of the mosquitoes [17]. Thus tracking of molecular, biochemical, and cellular events during *Plasmodium* developmental transition from one stage to another stage, is of particular interest. Several laboratory studies on mosquito-parasite interaction involving *P. berghei* or *P. falciparum*,

demonstrate that the developmental kinetics of the *Plasmodium* population is significantly altered, though the mechanism is not fully understood [18–20]. The last two decades of research highlights the crucial role of the tissue-specific mosquito immune system to control the parasite load, though the physiological relevance is yet to be investigated [21–24].

3. *Plasmodium* population dynamics and their immune regulation in the mosquito host

During *Plasmodium* development inside the mosquito host, the parasite population undergoes various bottlenecks. Previous investigations demonstrated that if a female mosquito takes ~1000 gametocytes through its infected blood meal, ~100 can be transformed into ookinetes, and among them, only 1–5 can successfully form oocysts. Furthermore, these survived oocysts will form millions of sporozoites, but only 19-20% can successfully invade the salivary glands for further transmission [25]. In refractory strains, not a single ookinete could transform into oocysts [26]. In general, a substantial loss of parasite population occurs at each developmental stage of the parasite, and this major parasite loss can be attributed to both human as well as mosquito components, which are harmful to *Plasmodium*.

The human component includes cytokines, complement protein, and reactive nitrogen species that are ingested along with the gametocytes during blood meal intake, and detrimental to the parasite within the midgut lumen of vector [26]. During the parasite transition through midgut epithelium, the mosquito mounts early immune response by increasing midgut nitration and activation of the signaling pathway. The nitration process modifies the ookinetes surface, and mark them to be recognized by the mosquito complement system when they emerge toward the basal side of the midgut [27]. Signaling pathways provide varying responses to various species of *Plasmodium*, such as the IMD pathway acts more efficiently against *P. falciparum* than *P. berghei*, and the Toll pathway is more responsive against *P. berghei*, and *P. gallinaceum* [28]. The proliferation of microbiota following blood meal also exacerbated the mosquito immune response, which in turn is detrimental to parasite development. Plasmodium parasite faces population bottlenecks throughout their development (in vertebrate as well as invertebrate host) but the mosquito midgut serves as the major site of extermination, where the number of parasites is minimal during the oocyst stage which makes it the most susceptible stage to identify molecular targets to disrupt the transmission [26]. Parallel to gut-immune interaction, several factors have been identified from mosquito hemocyte and salivary glands that interact with *Plasmodium sporozoites*; a bulk of literature is available on the mosquito innate immune system against *P. berghei* and *P. falciparum*, and therefore readers may refer to many excellent reviews [29–31]. Here we update the reports on the Mosquito-*P. vivax* interactions, and highlight their relevance for future implications.

4. Mosquito-*P. vivax* interaction

Undoubtedly, advanced omics technologies, especially genome sequencing and transcriptome analysis, has now become a basic method in living organisms for the assessment of genome-scale gene identification. The expression of large scale identified genes is currently being explored to decode the molecular complexity of *P. vivax* development in the vertebrate host. Earlier, a high-density tiling micro-array-based study showed the gene expression variation of *P. vivax* from human

and mosquito stages such as sporozoites, gametes, zygotes, ookinetes, and *in-vivo* asexual blood stages. Their comparison to *P. falciparum* and *P. yoelii* further reveals conserved and species-specific patterns highlighting the metabolic state of parasites growing within humans and identifies many orthologs of *P. falciparum* transcripts that are needed for exoerythrocytic development, which may also likely help in hypnozoite formation in the *P. vivax* [32].

4.1 *Plasmodium vivax* strategy to adapt in the mosquito *Anopheles stephensi*

The successful development of *P. vivax* within the midgut of a susceptible strain of *Anopheles stephensi* can be divided into two phases: pre-invasion (within midgut lumen) and post-invasion strategy i.e. development of oocyst stage which depends upon the nutrient availability within the host. During the pre-midgut invasion phase, *P. vivax* imparts an intricate mechanism to evade the mosquito immune response. It indirectly attenuates the mosquito immune response by dramatically suppressing the bacterial population, and whereas in the post- midgut invasion phase i.e. during the development of oocyst it modulates the expression of genes that are directly or indirectly involved with nutrition physiology to fulfill their nutritional requirement. We have limited information about the phases beyond oocysts maturation and their strategies to evade the mosquito immune system and promote their transmission.

4.1.1 Pre- invasion strategy of P. vivax

The midgut of Anopheles mosquitoes is housed by a complex and diverse community of bacteria, protozoa, fungi, etc. collectively referred to as the microbiota, and this microbiota is believed to shape the vector competency of mosquito. The gut bacteria of Anopheles mosquitoes adversely affect the *Plasmodium* infection [33, 34]. These tripartite interactions have been studied between the mosquito, its microbiota, and the *Plasmodium* parasites, but the precise relationship between the three remains unknown.

Numerous research reports have revealed that microbiota of specific bacterial species, particularly gram-negative bacteria, in many *Anopheles* species have an inhibitory effect on various *Plasmodium* species. The elimination of midgut bacteria through antibiotic treatment enhances oocyst load and parasite prevalence in different species of Anopheles. There are two mechanisms by which microbiota interfere with the *Plasmodium* development in the midgut lumen:-(i) indirectly by triggering the immune response of the mosquito (Imd Pathway) that guides the synthesis of AMP and other immune effectors that interferes with the development of parasites, and (ii) directly by certain bacterial species producing the metabolites that interfere with *Plasmodium* development and survival [33]. Recently, we have demonstrated that *P. vivax* plays a unique strategy to steer clear off the mosquito immune response during its pre-invasive phase, by dramatic suppression of the gut-bacterial population [35] (**Figure 1**). This study hypothesizes that the parasites outcompete the midgut microbiota presumably by scavenging the iron from the blood meal which is necessary for bacterial growth [35].

4.1.2 Post- invasion strategy of P. vivax (development of oocyst)

During the *Plasmodium* transit through midgut epithelium within the susceptible strain of Anopheles, some of the ookinetes successfully manage to escape the mosquito immune response [36], and reach the basal lamina of midgut to further differentiate into oocyst, and rests there nearly for two weeks. The sessile oocyst
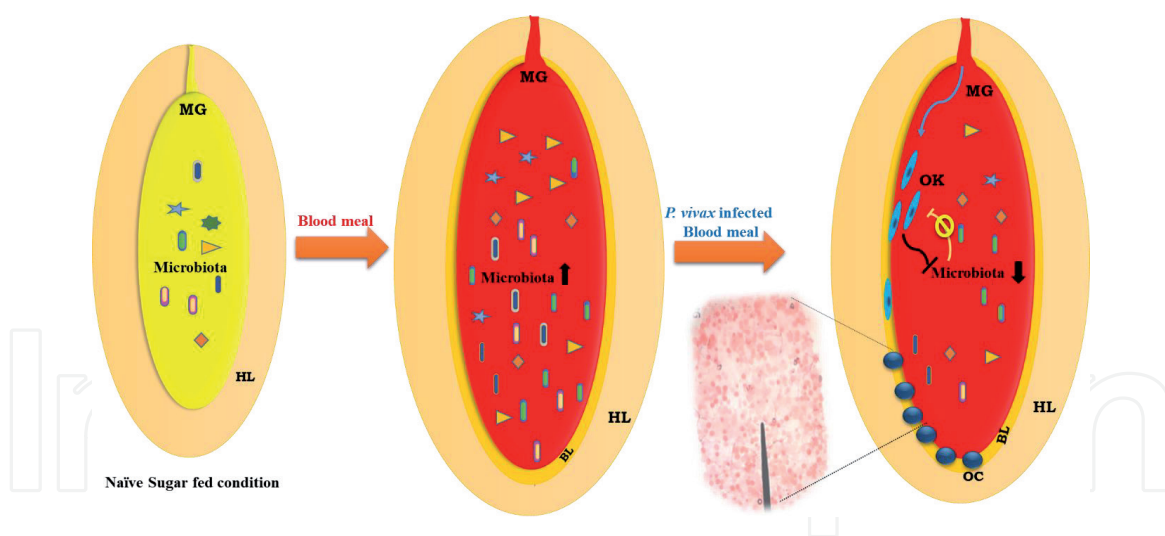
Figure 1.
Alteration of midgut microbiota proliferation by P. vivax. *Blood meal induces midgut microbiota proliferation within 24 hours. But during* P. vivax *infection, somehow this parasite restricts this microbiota proliferation after blood-meal, to avoid nutritional competition and immune defense exerted by the microbiota. This smart strategy of restriction helps the parasite to survive and proliferate better.*

stage is metabolically active, and follows an umpteen rounds of the nuclear division to transform into sporozoites. A single oocyst is capable of producing thousands of haploid sporozoites [37, 38]. Limited research has been undertaken on the underlying mechanism of *P. vivax* oocyst development (transition from a small oocyst of 7-8 µm to a large oocyst of 35-40 µm) in the mosquitoes. A few recent RNA-Seq analyses of *P. vivax* infected mosquitoes have been valuable to understand the ookinete and oocyst stage of *P. vivax* which reveals the alteration of several transcripts in the gut after 18 hours and 7 days post-infection in mosquito *Anopheles dirus* [39]. Notably, the authors identified several genes such as *Anoctamin 6* (ANO6; ADIR005670) and *Fibroblast Growth Factor* (FGF; ADIR008464), which may likely have immune regulation of *P. vivax* growth in the gut of the mosquito.

The parasite scavenges the nutrients from the host, and thus one of the main deciding factors of the infection outcome is likely dependent on the availability of nutritional resources of the host [40]. Our ongoing tissue-specific RNA-Seq analysis of *An. stephensi* infected with *P. vivax* oocyst identifies several unique sets of transcripts/genes, which have not yet find associated with any other *Plasmodium* infection. This study revealed the expression of genes involved in maintaining glucose homeostasis (*Trehalase*), nutrient transport (*Sterol Carrier* protein), energy, and nutrient homeostasis (*Folliculin*) during *P. vivax* infection [24]. We noticed that *P. vivax* infection modulates the *Trehalase* and *Sterol Carrier protein* expression in the midgut and salivary gland (SCP) for its own development and maturation. *Trehalase*, a glucosidase enzyme, catalyze the hydrolysis of disaccharide trehalose sugar into glucose units. Glucose is the main source of energy for the extensive proliferation of malarial parasites during both the blood and liver stages of malaria infection [41–44]. *Plasmodium* obtains the host glucose via hexose transporter. However, the role of sugar metabolism on *Plasmodium* infection in the mosquito vector remains poorly known. A multifold enriched expression of *Trehalase* transcript during early to late-stage oocysts in the gut as well as salivary glands, in addition to retrieval of *Plasmodium* hexose transcript in the midgut during oocyst stage, suggests that *Trehalase* may significantly contribute to hydrolyze the trehalose to provide glucose for the rapid proliferation of parasites, and also affect the reproductive capacity of adult female mosquito *An. stephensi* [45].

Similar to sugar requirement, *Plasmodium* also relies heavily on the host's cholesterol for its growth when maturing from small oocysts to large oocysts in

the gut. Since *Plasmodium* is incapable to synthesize *de-novo* cholesterol [46], and *P. vivax* infection induces a multifold expression of SCP after seven days of infection in the gut, likely indicates its role in cholesterol transport. Currently, there is no functional correlation exists between SCP and *Plasmodium* infection, however, with the current observation of SCP enrichment in the midgut as well as salivary gland, we propose that besides a possible role of supplying cholesterol to developing oocyst, it is possible that *As-SCP* may impart an anti-*Plasmodium* immune response, as increased lipid droplets have been shown in the midgut of *Ae. aegypti* during bacterial and viral infection [47]. *Folliculin* (FLCN) is a tumor suppressor protein associated with Birt-Hogg-Dube(BHD) syndrome [48, 49]. It is involved in many biological processes including vesicular trafficking, energy, and nutrient homeostasis, and monitors E-cadherin protein level [50, 51]. Late induction of *FLCN* in response to *P. vivax* infection (unpublished) suggests that it might also play an important role in maintaining the integrity of midgut epithelial cells during oocyst bursting or acquisition of nutrients by developing oocyst, though further studies needed to support this hypothesis.

4.2 *P. vivax* infection and immune strategy of the *Anopheles stephensi*

As described earlier, the parasite population undergoes several bottlenecks throughout their development inside the mosquito host. These bottlenecks are achieved because of the mosquito immune system [26]. Once the *Plasmodium* parasite transforms in the ookinete, midgut nitration modifies the parasite surface, which is then recognized by the hemocyte encoded pattern recognition receptors (like *TEP1*) circulating in the hemolymph [52]. Studies in the mosquito *An. gambiae* suggest that the complex of *LRIM1/APL1C* and *TEP1* bind to the parasite surface and activate the complement system, and in turn, the circulating hemocytes kill the parasite through cell lysis, phagocytosis, melanization, etc [53–55]. This whole phase is completed within 24 hours after infective blood meal uptake and is known as the
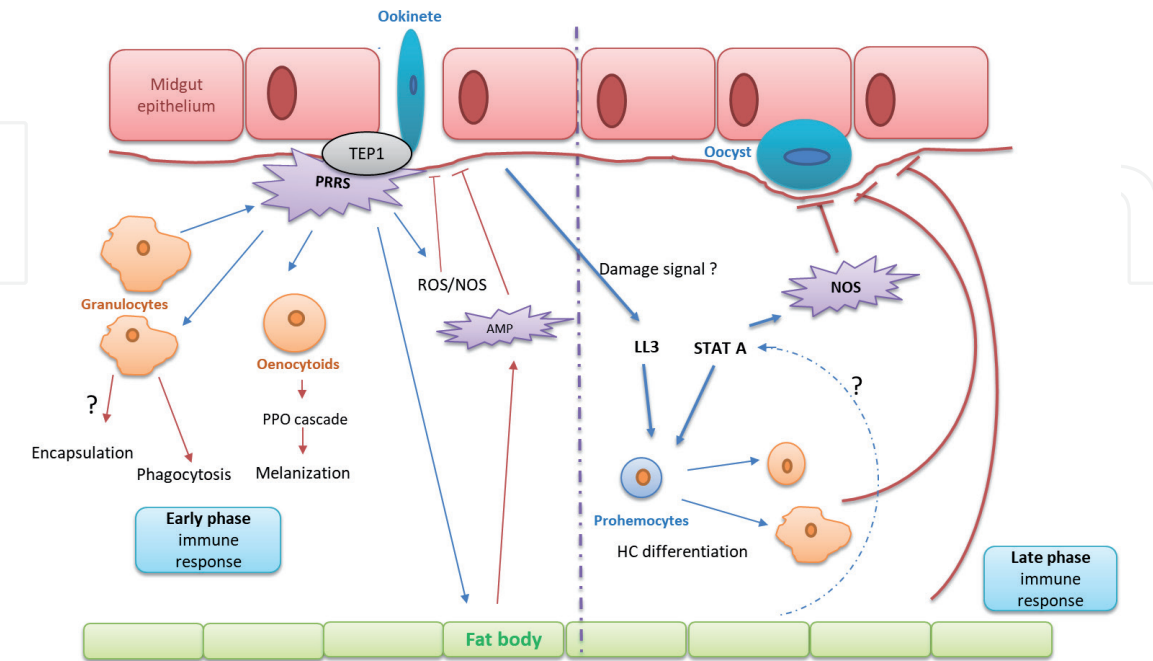

Figure 2.
Systematic representation of events occurring during early and late phase immunity in malaria parasite-infected mosquito: Once the ookinete invade the midgut epithelium, PRRs (pattern recognition receptors) like TEP1 recognize the pathogen and activate the complement system, which further triggers the hemocytes for phagocytosis, melanization, etc.

"early phase" immune response (**Figure 2**). Once the ookinetes reach the midgut epithelium, they get transformed into oocysts, and the immune system working against these transformed parasites is known as "late phase" immune response [56, 57]. Although very little is known about this phase but recent literature suggests that *LL3* mediated hemocyte differentiation, and *STAT* pathway activation, together helps in the restriction of the oocysts development [58]. Post oocysts maturation, millions of sporozoites evade the midgut lamina and circulate in the hemolymph, in order to reach and invade salivary glands for their successful transmission. Current literature suggests that among thousands of sporozoites only 19% can successfully invade the salivary gland, the rest are eliminated by the hemocyte mediated mosquito immune system [25]. But we have very limited information about this direct cell (hemocytes)-cell (free circulating sporozoites) interaction and elimination mechanism [29].

Altogether this information is restricted to the model organisms, and due to problems in culturing of *P. vivax* and extraction of hemocytes the exact species-specific interaction biology of this neglected parasite is still unknown [29]. As hemocytes play a crucial role in immune regulation, decoding the direct or indirect immune interactions between hemocytes and *P. vivax* parasite, will help us to figure out the parasite population control strategies of the mosquito hosts.

4.2.1 Hemocytes: the cellular immune army of the mosquito host

Mosquitoes have an open circulatory system, and hemocytes are the tiny blood cells circulating across the body reaching every mosquito tissue. These are the major immune elicitors working against a diverse range of pathogens [29]. Hemocytes are the core of the mosquito immune system which can induce both cellular as well as humoral immune responses [30, 59, 60]. Mosquito hemocytes population can be discriminated on the basis of their anatomical location (circulatory and sessile), DNA content (euploid and polyploid), morphology, and functions (granulocytes, oenocytoids, and prohemocytes) [61–63]. Granulocytes are the phagocytic cells, which engulf the invaded parasite and kill them by lysozyme activity [64, 65]. Oenocytoids are the producers of the *Pro-phenoloxidases*, the rate-limiting enzyme of the melanization pathway [66]. Melanization is the systematic enzymatic process, which ultimately produces the melanin protein. When a foreign invader infects the mosquito, hemocytes cover the parasite in the melanin envelop, which will cut-off the parasite from the outside environment, nutrition, and also induces oxidative stress which results in the killing of the parasite. Prohemocytes are considered as the progenitor cells, which produce granulocytes and oenocytoids, although the actual function is not known yet about these tiny cells [64, 67]. Previous literature illustrated various hemocyte encoded molecules, like *TEP1*, *FBN30*, *LRR3*, etc. are vital for the early and late phase immune responses [55, 68–71]. Researchers have also successfully tracked the involvement of phagocytosis and melanization events for the removal of parasites [17]. But we do not have much information about the direct cell–cell interaction of the hemocytes and *P. vivax* free-circulating sporozoites (*fcSPZ*).

Recently we conducted a transcriptome based study, to understand that how hemocytes control the *P. vivax* free circulatory sporozoites (*fcSPZ*) population before salivary invasion [24]. Here we found that hemocyte encoded transcripts undergo a major shift during *P. vivax* infection. A detailed comparison of the *P. vivax* infected and uninfected hemocyte transcriptomes revealed that transcripts of organelle organization and riboprotein complex biogenesis have exclusively emerged during *P. vivax fcSPZ* infection. Altogether these findings suggested that the hemocyte population undergoes dynamic changes i.e., differentiate and increase the population in response to the *fcSPZ*. Through the immune database comparison,

we found that AMPs like *Defensin and Gambicin* were exclusively induced when *fcSPZ* were circulating in the hemolymph. These findings were further validated by the real-time based experiments and depicted that *Defensin3* and *Gambicin* may likely play a crucial role during *P. vivax* late-phase immune function against *fcSPZ* infection. Hence, conclusively current findings illustrate that hemocytes rapidly proliferate and impart humoral immune responses against the parasite to limit the *fcSPZ* population before salivary gland invasion (**Figure 3**).

Apart from global transcriptomic changes undergone by the hemocyte population to manage *P. vivax* infection, we also found the species-specific molecular differences among the hemocyte encoded immune transcripts. *FBN9* which was previously considered as the potent anti-*Plasmodium* molecule and showed multi-fold upregulation during *P. berghei/ P. falciparum* infection [71, 72] was found to be downregulated during *P. vivax* infection. Novel molecules like *FREP12* and *FREP50* were predicted to be involved in the clearance of *P. vivax* sporozoites. Furthermore, storage proteins like *ApolipophorinIII*, *Hexamerin* were also found to be highly induced during *P. vivax* oocysts development, which further supported the previous evidence of host nutrient scavenging by the maturing oocysts [73–75].

4.2.2 Mosquito salivary glands: Gatekeeper of entry and exit for the parasite

The salivary glands are the crucial organ for the development and transmission of the *Plasmodium* to a vertebrate host. Salivary glands are paired epithelial organs that are located in the thorax, and consist of three lobes namely, two lateral and one median, where each lateral lobe is comprised of proximal and distal lobes [76, 77]. The proximal portion of the female glands produces enzymes involved in sugar metabolism, where distal lobes are shown to play roles in blood meal acquisition, *Plasmodium* invasion, and transmission. Although, studies suggest that only 10–20% hemolymph circulating sporozoites, manage to invade the salivary glands, however, the mechanism of this drastic reduction of 80–90% sporozoites is poorly
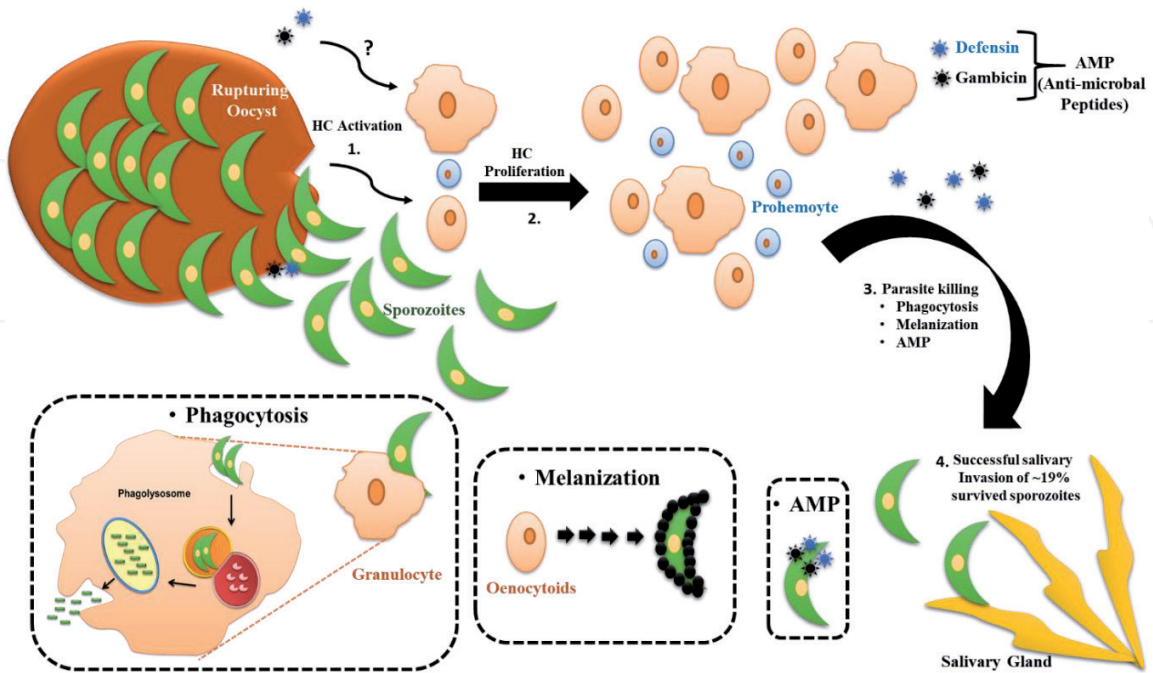

Figure 3.
Direct interaction of free-circulatory sporozoites and hemocytes. Post oocyst maturation, sporozoites circulate freely in the hemolymph, in order to reach salivary glands for further transmission. But oocyst rupture triggers the mosquito immune system and activates the hemocyte proliferation, which leads to the sporozoite clearance by phagocytosis, melanization, and AMP (Defensin and Gambicin) production. Although the role of AMPs in hemocyte activation is still unclear.

known [25, 78]. Accumulating evidence highlights that sporozoite invasion into the glands is mediated by salivary specific receptor-ligand interactions [18, 79].

The sporozoites must leave (egress) the oocyst after maturation to invade the salivary gland and to be transmitted to the next vertebrate host. The egress of sporozoite is mediated by a protease named *Cysteine protease* (ECP1) which ruptures the oocyst [80, 81]. The sporozoites are released into the hemolymph and carried to the salivary glands by the circulation of hemolymph in an anterior direction from the abdomen to head, and facilitate the sporozoite invasion to the salivary gland [82]. The salivary gland epithelium forms a physical barrier that pathogens must cross, and *Plasmodium* parasites are evolved with unique proteins that drive invasion by first binding to the salivary gland specific surface receptors [83]. The salivary invasion process completion occurs in two stages, where first, sporozoite binds to invade the salivary gland basal lamina; and second, then interacts with the plasma membrane of the epithelial cells favoring sporozoite internalization. During the invasion, sporozoites attach and invade the distal and medial lobe of the salivary glands, and this attachment and invasion are highly specific to the nature of *Plasmodium* species [84, 85].

Empirical evidence showing that the salivary glands serve as an active immune organ is largely lacking, except some studies highlighting that a *Serine Protease Inhibitor* (SRPN6) produced in the salivary epithelium limits gland invasion by *Plasmodium sporozoites*, and thus *SRPN6* serves as an important salivary invasion immune-marker [86]. Several putative salivary encoded factors such as *Saglin*, *CSP* binding proteins which effectively binds with sporozoites surface antigens such as *TRAP*, *CSP* are well known salivary receptors for sporozoites invasion [87–91]. However, several other salivary factors such as *Plasmodium Responsive Salivary1* (PRS1), *ESP*, *Peptide-O-xylosyl Transferase 1* (OXT1) have also been identified to play a crucial role in parasite invasion of both midgut and salivary glands [92–95]. Once inside the salivary glands, the parasite undergoes transcriptional reprogramming before its transmission to the next mammalian host.

Transmission of many viral and protozoan parasites to a vertebrate host requires their salivary injection with the mosquito saliva during blood-feeding, and thus the migration of sporozoites needs duct for the continuation of the life cycle. Mosquito saliva has a pleiotropic property such as anti-hemostatic, vasodilator, or anti-inflammatory properties and immune modulators, and basic function to facilitate blood-feeding [96–98]. However, saliva proteins can also have an indirect impact on pathogen development and transmission. For example, a recent study in mosquito *An. gambiae* shows that mosquito saliva proteins such as *AgTRIO* and *mosGILT* serve as an important mediator of the transmission of *P. falciparum*, and inhibition of this protein can reduce the parasite burden in the human host [99, 100]. Although, a major study on salivary-sporozoites interaction is restricted to *P. berghei* and/or *P. falciparum*, however, very limited information is available on the salivary-*P. vivax* interaction.

A comparative RNA-Seq analysis of uninfected and *P. vivax*-infected mosquito salivary glands suggests that salivary transcripts undergo substantial changes during *P. vivax* infection. The maturation of sporozoite seems to coincide with the change in gene expression essential for invasion and transmission. Findings of several classes of immune proteins such as *Heme-peroxidase*, *FADD*, *Gambicin*, *GNBP*, and multiple family proteins of *Serine proteases*, and *SCRC* in the *P. vivax* sporozoites invaded salivary glands highlighted their anti-*Plasmodium* immune role of salivary glands. The transcriptome of the infected salivary glands also revealed that *P. vivax* infection decreased the expression of apyrase significantly which suggests that *P. vivax* interferes with salivary secretion before probing and feeding to ensure their delivery into the next human host. These findings offer valuable new insights into the biology of malaria parasites. Manipulating tissue-specific immuno-physiology of the mosquitoes may halt the *Plasmodium vivax* development and hence the transmission (**Figure 4**).
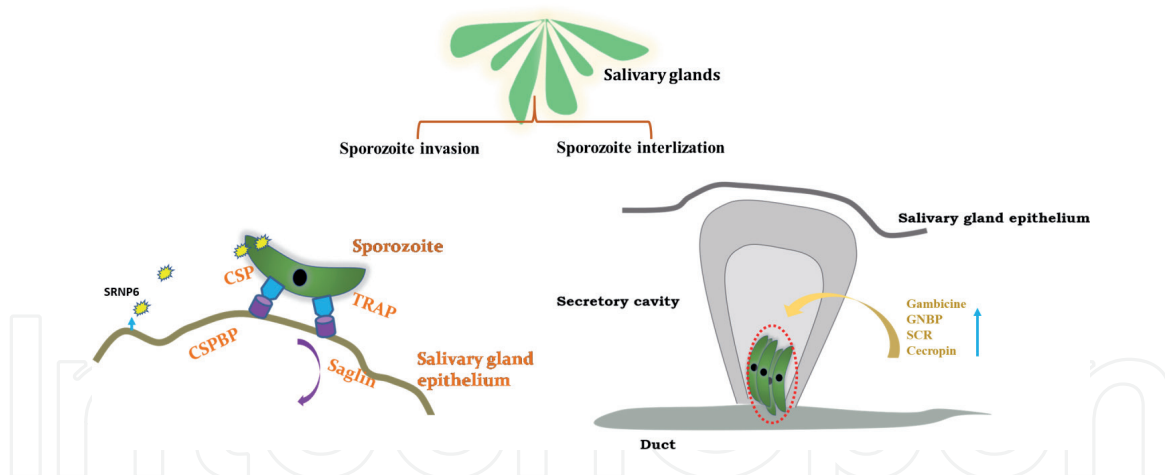
Figure 4.
*Proposed hypothesis of salivary gland-*Plasmodium *interaction and transmission: Free circulating sporozoite in the hemolymph recognize and attach to the basal lamina of the salivary gland receptor-ligand interaction;(1) initial attachment of the sporozoite mediated by interactions of carbohydrate residues on the basal lamina with a parasite CS, SGS1, MABEL. CSP binding protein and* Saglin *bind with CSP and TRAP respectively are an important component of salivary gland invasion. (2) Sporozoite internalization: After invasion sporozoite passes into the secretory cavity and sporozoites begin to assembly there as a large bundle form. Within the salivary duct component of the mosquito immune responses* Gambicin, Cecropin, GNBP, *and* SCR *family members presumably act upon sporozoite and limit the number.*

5. Conclusion

Plasmodium and mosquito host both are involved in the dynamic molecular relationship, where parasite tries to dodge the host immune system and utilize its nutrients for their successful proliferation/ transmission. On the contrary, the mosquito host immune system tries to restrict the parasite development and eliminate the remnants. During this ultimate battle, some host species defeat the parasite through its active immune system and become resistant but in others, the parasite smartly manipulates the host system and defends itself for successful transmission.

P. vivax is one of the neglected parasites which successfully manipulated the host system for its efficient transmission. *P. vivax* suppresses the microbiota proliferation to avoid nutritional competition as well as early immune responses. Different nutrient transport proteins like *Trehalase*, *Sterol Carrier*, *ApolipophorinIII*, etc. were modulated by the parasite for fulfilling its nutritional requirements. But still, mosquito hosts also developed species-specific immune effector molecules like *FREP50*, *FREP12*, *LRIM17*, etc. to block the parasite development. Likewise finding salivary-specific factors such as *Heme-peroxidase*, *SP24D* that are crucial to sporozoite invasion and survival, may further help to halt the progression of *Plasmodium* development and malaria transmission.

In summary, future functional exploration of the novel *P. vivax* specific host factors, will help in the development of transmission-blocking vaccines and the generation of new intervention techniques or modify current ones.

Acknowledgements

Conflict of interest

No competing interests were disclosed.

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