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Human Erythrocyte Remodelling by *Plasmodium falciparum*

Xue Yan Yam^{1,2}, Alassane Mbengue¹ and Catherine Braun-Breton¹

¹University Montpellier II, CNRS UMR5235, University Montpellier I,
Dynamique des Interactions Membranaires Normales et Pathologiques,

²School of Biological Sciences, Nanyang Technological University, Singapore,

¹France

²Singapore

1. Introduction

Making the erythrocyte its home for 48 hours has important consequences for the human malaria parasite *Plasmodium falciparum*. Indeed, erythrocytes are terminally differentiated cells that lack a nucleus as well as intracellular organelles, are thus unable to endocytose or exocytose macromolecules, and have lost several membrane transport activities upon differentiation. Consequently, the parasite has to deeply remodel its host cell, from the very beginning to ensure its entry into the red blood cell, throughout its growth and multiplication to fulfil its needs for extracellular nutrients, and to the very end of its intra-erythrocytic development with the parasite-induced opening and curling of the red cell membrane leading to the dispersion of newly formed merozoites into the blood flow. The most spectacular, and first reported, modifications of the red blood cell membrane induced by *P. falciparum* are electron dense protrusions named knobs and consisting of parasite proteins exported to the red cell membrane and sub-membrane skeleton where they eventually interact with host cell proteins. Knobs are directly related to the severity of *falciparum* malaria because they mediate adherence of infected erythrocytes to the microvasculature endothelium. More recent studies have revealed that the parasite might export several hundreds of proteins, as well as membrane compartments, to the red cell and divert enzymatic and structural host proteins to make the erythrocyte a suitable environment for its growth. In the last decade, remodelling of its host cell by *Plasmodium falciparum* has become an important and growing field of research. In this review, we will describe the current stage of knowledge concerning red blood cell remodelling by *Plasmodium falciparum* and the role of these parasite-induced modifications for its growth and survival.

2. Early modifications of the red blood cell

Apicomplexan parasites share a conserved mode of invasion by actively entering their host cell with the formation of a specialised junction with the host cell membrane and the establishment of the parasite inside a self-induced parasitophorous vacuole (Aikawa *et al.*, 1978). Initial attachment of the parasite to the host cell surface results from low-affinity

reversible interactions (Dvorak *et al.*, 1975) and induces the sequential discharge of two types of apical secretory organelles: 1/ the micronemes, small secretory organelles underlying the parasite's apical pole and providing a variety of adhesive proteins and 2/ the pear-shaped rhoptries providing rhoptry neck proteins that, in collaboration with microneme proteins establish a junction between the invading parasite and its host cell membrane named the "moving junction" (Aikawa *et al.*, 1978). For malaria parasites, initial attachment triggers waves of deformation of the red cell membrane (Figure 1) (Gilson & Crabb, 2009), that cover the merozoite and might facilitate the formation of the junction between the merozoite's apical pole and the host cell membrane. Noteworthy, to form this junction, the parasite exports to the host cell membrane its own receptor, Ron2, for the parasite surface ligand AMA1 (Besteiro *et al.*, 2009). Additional rhoptry neck proteins, Ron4, Ron5 and Ron8, are secreted to the cytosolic face of the host cell plasma membrane and participate in the junction formation that provides the parasite an anchoring to the host cell membrane supporting forward motion of the parasite with the apical pole leading the way (Besteiro *et al.*, 2011). This active penetration promotes invagination of the host cell plasma membrane with the moving junction acting as a sieve excluding host cell integral membrane proteins from the nascent parasitophorous vacuole membrane (PVM) while some glycosylphosphatidylinositol (GPI)-anchored and lipid raft-associated proteins enter the vacuole (Aikawa *et al.*, 1981; Atkinson *et al.*, 1988; Dluzewski *et al.*, 1989; Dluzewski *et al.*, 1988). Noteworthy, the malarial parasite seems to exploit glyco-sphingolipids and cholesterol enriched microdomains of the erythrocyte membrane known as lipid rafts for invasion: this is supported by the evidence that the merozoite infection is halted following disruption of raft-cholesterol using the cholesterol depleting agent, methyl- β -cyclodextrin (MBCD) (Samuel *et al.*, 2001). In addition, *P. falciparum* entry is blocked by lidocaine hydrochloride, a local anaesthetic agent reversibly disrupting the lipid rafts without altering the cholesterol content of the erythrocyte membrane (Koshino & Takakuwa, 2009). A proposed mechanism for this is that the disruption of rafts alters an erythrocyte raft hetero-trimeric guanine nucleotide-binding protein-mediated signal transduction pathway that induces the phosphorylation of sub-membrane skeletal proteins (Kamata *et al.*, 2008). These phosphorylations can modify the mechanical properties of the erythrocyte membrane [reviewed in (Zuccala & Baum, 2011)] and favour membrane invagination. The major and raft-associated erythrocyte membrane protein Band 3 appears to be phosphorylated on tyrosine residues upon invasion (Pantaleo *et al.*, 2010). This phosphorylation should result in the clustering of Band 3 and thus be important for parasite entry by de-connecting Band 3 from the erythrocyte sub-membrane skeleton (Ferru *et al.*, 2011). In addition, G-protein coupled signalling through the β 2-adrenergic receptor, has also been shown to regulate the parasite invasion efficiency (Harrison *et al.*, 2003) and growth (Murphy *et al.*, 2006a). All these studies strongly imply that erythrocyte rafts are functionally exploited for parasite invasion and also serve as a platform for signalling events to take place.

The biogenesis of the PVM is dynamic and has not been completely resolved. Immunoelectron microscopy studies have provided evidences that apical organelles of the merozoite contain and release into the erythrocyte lipidic lamellar materials which could participate in the PVM expansion (Bannister & Mitchell, 1989; Bannister *et al.*, 1986; Mikkelsen *et al.*, 1988). Additionally, as described in (Dluzewski *et al.*, 1995) the PVM does not contain lipids solely from the host cell membrane as the surface area of newly infected erythrocytes had not evidently decreased in size, suggesting the contribution of lipids from

the parasite itself. On the other hand, exchange of lipids between the parasite and erythrocyte membrane have also been reported (Hsiao *et al.*, 1991) and studies using fluorescent lipophilic probes revealed that the PVM does contain lipids from the host cell membrane (Haldar *et al.*, 1989; Ward *et al.*, 1993). All these data illustrate that the biogenesis of the PVM appears to have relative contributions from both parasite- and host cell erythrocyte-derived lipids. In addition, a lipid raft based biogenesis of the PVM has been proposed (Hiller *et al.*, 2003).

Although there is no formal proof for a role of rhoptry bulb proteins in the formation of the parasitophorous vacuole, their association to the parasitophorous vacuole membrane suggests that they participate in early stages of its biogenesis. However, direct evidences have been obtained in *T. gondii*, showing that rhoptry proteins, particularly protein kinases and phosphatase, secreted to the parasitophorous vacuole membrane or host cell nucleus serve as effectors, and constitute major virulence factors that counteract the immune response of the host (Behnke *et al.*, 2011; El Hajj *et al.*, 2007; Gilbert *et al.*, 2007; Saeij *et al.*, 2006). The Band 3 phosphorylation on tyrosine residues mentioned above might be induced by a, yet unidentified, secreted parasite protein kinase or by the activation of an erythrocyte tyrosine-kinase.

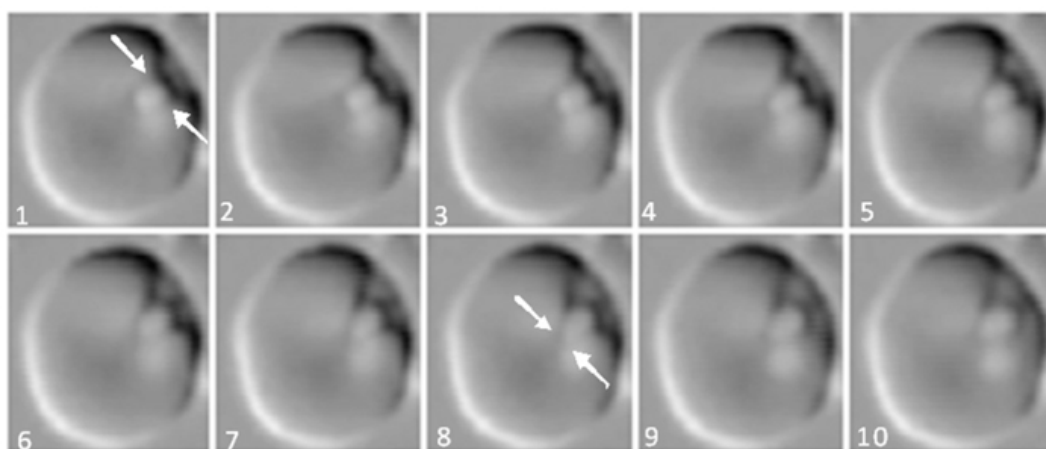


Fig. 1. Erythrocyte membrane deformations generated by a *P. falciparum* merozoite. The merozoite glides on the surface of a red blood cell membrane prior to entrance (Time lapse of 0.5 s between each frame). The membrane is deformed by the strength of adhesion. The adhesion site is transferred from the back of the merozoite (frame 1, white arrows) to its apical pole (frame 8, white arrows). High speed live imaging with the participation of Magali Roques and Manouk Abkarian.

Host proteins also participate in the development of the parasitophorous vacuole since the selective vacuolar uptake of several DRM-associated erythrocyte membrane proteins has been reported, including both transmembrane and GPI-anchored proteins (Lauer *et al.*, 2000; Murphy *et al.*, 2004; Bietz *et al.*, 2009). However, not all proteins derived from the erythrocyte DRMs are recruited to the PVM, suggesting that the recruitment does not depend only on their DRMs association. The moving junction is likely playing a central role in this selection process that might participate in changing the physical properties of the erythrocyte for efficient parasite entry (Mordue *et al.*, 1999; Murphy *et al.*, 2004). Interestingly, dematin, an erythrocyte sub-membrane skeleton binding protein, was also

recently found to be internalized by the parasite (Lalle *et al.*, 2011). The biological functions of these internalized proteins remain enigmatic and further studies are necessary to determine whether internalization of these proteins is essential for the parasite survival and in maintaining the stability of the vacuolar environment. However, both Band 3 tyrosine-phosphorylation and dematin internalisation participate in a parasite-induced fragility of the red cell membrane likely required for efficient merozoite entry (Ferru *et al.*, 2011; Khanna *et al.*, 2002) while the Ring-infected Erythrocyte Surface Antigen (RESA) released by the merozoite into the red blood cell upon invasion stabilizes spectrin tetramers and confers the infected erythrocyte enhanced resistance to mechanical and thermal degradation (Pei *et al.*, 2007). Noteworthy, the binding of RESA to spectrin tetramers also confers the newly infected erythrocyte resistance to further invasion (Pei *et al.*, 2007).

Moreover, using *Plasmodium knowlesi* parasites, Torii and collaborators have observed the release of the dense granule contents into the lumen of the parasitophorous vacuole and the concomitant invagination of the PVM adjacent to the released contents (Torii *et al.*, 1989). These results suggested that the dense granules, another type of apical secretory organelles of the merozoite, play a role in forming finger-like channels extending into the surrounding erythrocyte cytoplasm. Numerous studies using primarily *Toxoplasma gondii* parasites but also *Plasmodium falciparum* showed that the released dense granule contents transform the parasitophorous vacuole into a metabolically active compartment [reviewed in (Mercier *et al.*, 2005)].

3. Living within the parasitophorous vacuole

The intracellular parasite living in the vacuole acquires nutrients by uptake from the host cell cytosol and extracellular milieu, hence the PVM has dual roles: (i) protect the parasite from extracellular harmful substances and (ii) facilitate nutrients access to parasite needs (Lingelbach & Joiner, 1998). Upon parasite growth and parasitophorous vacuole enlargement, extensions from the PVM form membranous whorls and loops and tubular elements projecting to the host cell periphery without fusing with the red blood cell membrane. These PVM extensions form an interconnected network of tubular and vesicular membranes known as the tubovesicular network (TVN) (Atkinson & Aikawa, 1990; Elmendorf & Haldar, 1994; Grellier *et al.*, 1991). Inhibition of the parasite sphingomyelin synthase activity, localised to the TVN (Elmendorf & Haldar, 1994), by dl-threo-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) arrested the assembly of the interconnected TVN network and resulted in the blockage of the delivery of extracellular nutrients to the parasite (Lauer *et al.*, 1997), indicating the importance of TVN in nutrients import for the intracellular parasite. In addition, using a comparative transcriptomic analysis of PPMP-treated *P. falciparum* infected erythrocytes, Tamez and colleagues have identified erythrocyte vesicle protein 1 (EVP1), a parasite protein implicated in the maintenance of the TVN for nutrients import (Tamez *et al.*, 2008). Furthermore, van Ooij *et al.* have shown that the exported protein PfC435 localises at vesicles proposed to connect the PVM and TVN and to be involved in the TVN formation (van Ooij *et al.*, 2008). There are most probably more proteins involved in the formation of this network, which call for further investigations.

3.1 Nutrient uptake and induction of new permeability pathways

The trophozoite growth is accompanied by extensive digestion of the red blood cell cytoplasm. However, it is not sufficient to provide the parasite all the nutrients it needs to sustain its growth: for example, haemoglobin does not contain isoleucine, and several other amino acids such as glutamate, methionine, cysteine and proline are under represented; in addition, the red blood cell has lost many membrane transporter activities upon differentiation from reticulocytes, thus limiting the parasite's access to extracellular nutrients. Consequently, the intra-erythrocytic growth of the parasite depends on its ability to efficiently uptake a range of essential nutrients from the extracellular milieu through the host cell membrane to the TVN (Lauer *et al.*, 1997). This is achieved by both the use of constitutively active host cell transporters and by the creation of new permeability pathways (NPPs) in the host cell membrane [reviewed in (Kirk, 2001)].

The permeability to a wide range of physiologically relevant solutes is newly detected in the infected erythrocyte membrane at the trophozoite stage of the parasite (Ginsburg *et al.*, 1985; Homewood & Neame, 1974; Staines *et al.*, 2001). They might originate both from parasite-encoded transporters that are delivered to the host cell membrane, and from the modulation of endogenous transporters of the erythrocyte by parasite-encoded proteins. Indeed, the NPPs depend on parasite proteins either as components of the NPPs or as modulators of endogenous erythrocytic transporters as first demonstrated by their re-appearance in intact infected erythrocytes following inactivation by chymotrypsin treatment and further suspension in a chymotrypsin-free medium (Baumeister *et al.*, 2006). NPPs re-appearance depends on the parasite viability and ability for protein secretion. Nguitragool and collaborators have recently determined that the parasite Clag3 proteins, exported to the red blood cell membrane, contribute to a novel ion channel with unusual selectivity and conductance properties (Nguitragool *et al.*, 2011). Moreover, several parasite protein-kinases are exported to the erythrocyte cytosol (Nunes *et al.*, 2007) that might modulate the activity and specificity of pre-existing inactive membrane transporters. A specific and high affinity interaction of serum albumin with the surface of infected erythrocytes has also been shown to stimulate anion conductance in the host erythrocyte membrane, thus clearly illustrating the participation of both parasite and host factors in the activation of NPPs (Duranton *et al.*, 2008).

3.2 Protrusions at the cell surface mediate sequestration of *P. falciparum*-infected erythrocytes

The parasite-induced changes at the red blood cell membrane described above, would end up in a very efficient splenic removal of infected erythrocytes from the blood circulation if the parasite had not been able to confer adhesive properties to its host cell. Indeed, cytoadherence of *P. falciparum*-infected erythrocytes to the microvasculature endothelium has been observed which results in their sequestration at the mature trophozoite and schizont stages of the parasite. This cytoadherence is mediated by the parasite adhesin, PfEMP1, exposed at electron-dense protrusions of the erythrocyte surface, referred to as knobs (Baruch *et al.*, 1995; Fairhurst & Wellem, 2006; Fremont & Miller, 1975). The key player in knobs formation is the knob-associated histidine-rich protein (KAHRP or HRP-1) as absence of this protein results in knobless infected erythrocytes (Crabb *et al.*, 1997; Kilejian, 1979). In addition, the C-terminal region of this protein has been shown to be

essential for the formation of functional knobs (Rug *et al.*, 2006). The knobs-mediated cytoadherence of *P. falciparum*-infected erythrocytes and its implication in the pathogenesis of severe malaria [reviewed in (Rowe *et al.*, 2009)] have been the subject of numerous studies and reviews and will only be shortly described here with special focus on the molecular organization of knobs.

As the parasite matures from trophozoite to schizont, the knobs increase in density (from 10-35 to 45-75 knobs/ μm^2) and eventually cover the entire red blood cell surface while their size varies inversely from 160-110 nm to 70-100 nm in diameter (Gruenberg *et al.*, 1983). Their formation implies dynamic changes to the erythrocyte membrane and sub-membrane skeleton, which involve redistribution and organization of constituents from both parasite and host cell origin. The knob-associated histidine-rich protein (KAHRP) self-aggregates (Kilejian *et al.*, 1991) and anchors the carboxy-terminal domain of PfEMP1 to the erythrocyte sub-membrane skeleton at the actin-protein 4.1-spectrin junction (Waller *et al.*, 1999; Waller *et al.*, 2002). In addition, extractability data strongly suggest that other red blood cell membrane-associated proteins are implicated because the insertion of PfEMP1 in the red blood cell membrane seems to rely more on protein-protein interactions than protein-lipid interactions (Papakrivos *et al.*, 2005). Indeed, beside KAHRP, other parasite and erythrocyte proteins affect the amount and distribution of PfEMP1 at the red blood cell surface (Allred *et al.*, 1986; Fairhurst & Wellem, 2006). Many studies have contributed to provide an integrated model of the knob structure [reviewed in (Maier *et al.*, 2009)], implicating erythrocyte cytoskeletal components such as spectrin, ankyrin and actin and thus altering the physical properties of the erythrocyte by increasing its rigidity and adhesiveness (Pei *et al.*, 2005). However, while the 5' repeat region of KAHRP is required for the knob protrusion (Rug *et al.*, 2006), the precise interactions at the red blood cell membrane and sub-membrane skeleton causing protrusion of the red blood cell plasma membrane still need further investigations.

Besides the TVN and knobs, many other parasite-induced changes in the red blood cell and different populations of vesicular-like membrane compartments have been observed in the infected erythrocyte which might be implicated in the trafficking of nutrients, lipids and parasite-encoded proteins within the host cell (Grellier *et al.*, 1991; Hanssen *et al.*, 2010; Külzer *et al.*, 2010; Tamez *et al.*, 2008).

4. The Maurer's clefts, a novel secretory compartment transposed in the host cell cytosol

Within tens of seconds after merozoites entry and sealing of the parasitophorous vacuole, the erythrocyte membrane deforms from its biconcave disc shape to an echinocyte shape and returns to its normal state after several minutes (Gilson & Crabb, 2009) (Figure 2). This echinocytosis might be the result of the invagination of the red cell membrane and changes to the host cell cytoskeleton (Pantaleo *et al.*, 2010) or induced by an efflux of potassium and chloride ions (Gilson & Crabb, 2009). In addition, these fluctuations of the red cell membrane might correlate with 1) the insertion of lipids in the external leaflet of the host cell membrane likely secreted with the rhoptry content upon invasion that would result in increasing the area of the red cell membrane external leaflet and explain the formation of spicules; 2) modifications of the erythrocyte membrane / sub-membrane skeleton interactions upon parasite entry. Two modifications of the erythrocyte Band 3 necessary for

efficient parasite entry have been observed: cleavage by the rhoptry protease Pfgr76 resulting in increased uptake of phospholipids by the red cell membrane (Braun-Breton *et al.*, 1992; Roggwiler *et al.*, 1996) and hyper-phosphorylation resulting in the dissociation of Band 3 interactions with the cytoskeleton (Ferru *et al.*, 2011; Pantaleo *et al.*, 2010). Such a detachment is concordant with the spectacular echinocytic and transient shape transformation of the erythrocyte after invasion. At approximately the time of resumption of the erythrocyte to its normal shape, parasite-induced membranous compartments termed Maurer's clefts are present and observed to be scattered within the host cell cytoplasm (Gruring *et al.*, 2011) before predominantly residing in close vicinity of the erythrocyte periphery.

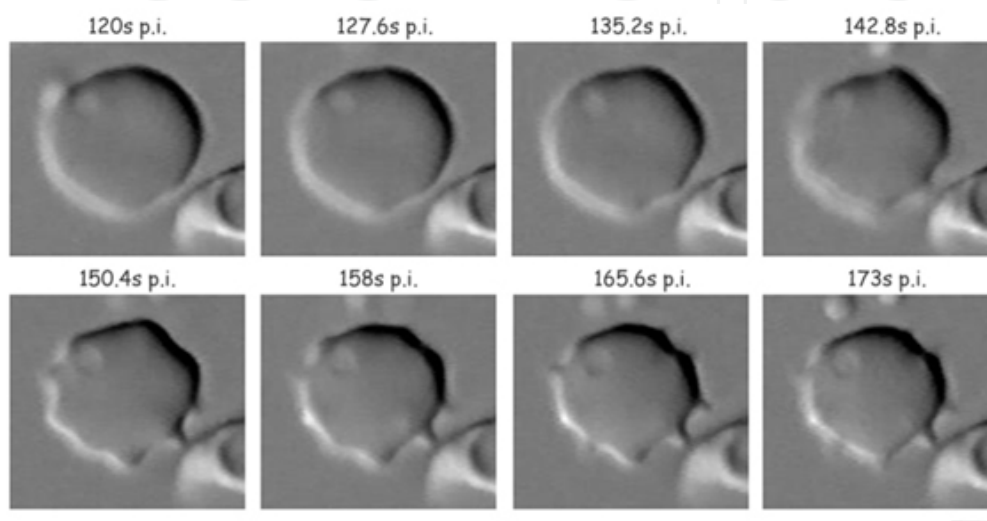


Fig. 2. Red blood cell deformations following the entry of a *P. falciparum* merozoite. Change of the erythrocyte shape started about 2 min following entry of a *P. falciparum* merozoite, with the formation of membrane spicules and generating an echinocyte morphology. The time scale post-invasion is indicated on each snap-shot. High speed live imaging with the participation of Magali Roques and Manouk Abkarian

In 1902, the German physician Georg Maurer has described a peculiar dotted staining pattern in the cytoplasm of *P. falciparum*-infected erythrocytes stained with Giemsa. Georg Maurer has provided a complete and in-depth description of these structures that were then named Maurer's clefts in his honour (Lanzer *et al.*, 2006). The significance of the discovery of Maurer's clefts remained unrecognized for almost a century till presently it has become one of the focuses of intense malaria research concerning their morphology, biogenesis and functional roles.

4.1 Morphology of Maurer's clefts

Trager and co-worker were among the first researchers to resolve the dotted pattern as long, narrow, slender single membrane surrounded clefts (Trager *et al.*, 1966). Ultra-structural studies showed that Maurer's clefts have a distinct morphology as stacks of flattened lamellae of long slender membrane of about 0.2-0.5 μm in length with translucent lumen and electron dense coat of variable thickness (60-100 nm) located predominantly at the erythrocyte membrane periphery as the parasite matures (Etzion & Perkins, 1989; Wickert &

Krohne, 2007). 3D reconstructions have added another level of complexity to the organization and structure of Maurer's clefts. The simplest form of Maurer's clefts is a single, disc-shaped cistern localized beneath the erythrocyte membrane with height and width of at least 500 nm. Maurer's clefts with more complex morphology are formed by small stacks of parallel cisternae with height of 650-800 nm and width of 300 nm (Wickert *et al.*, 2004; Wickert & Krohne, 2007). In addition, electron tomography, a technique collecting a series of images titled at different angles and tomography reconstruction of the aligned electron micrographs to generate a 3D model, has been recently applied to obtain a spatial image of Maurer's clefts (Hanssen *et al.*, 2010; Hanssen *et al.*, 2008b; Henrich *et al.*, 2009; Tilley & Hanssen, 2008). Hanssen and colleagues have revealed the 3D complexity of Maurer's clefts with convoluted flotillas of flattened disc-shape structures with translucent lumen and a more electron dense coat; some regions are decorated with surface nodules each of ~25 nm in diameter (Hanssen *et al.*, 2008b; Tilley & Hanssen, 2008). Differences were also observed in the complexity of the clefts between different *P. falciparum* strains. In D10 strain, more than 60% of Maurer's clefts have more than two cisternae, while in 3D7, only 10% show such complex organization (Frischknecht & Lanzer, 2008; Hanssen *et al.*, 2008b), suggesting that additional studies with a range of field and laboratory strains are needed to have a complete overview of the Maurer's clefts morphology (Hanssen *et al.*, 2008b).

4.2 Biogenesis of the Maurer's clefts

The biogenesis of Maurer's clefts still remains an open area of research. Wickert and colleagues proposed that Maurer's clefts form a continuous network from the PVM/ TVN with Maurer's clefts arising at one or more sites from the PVM/ TVN membrane and extending across the host cell cytoplasm to the inner leaflet of the erythrocyte plasma membrane (Wickert *et al.*, 2004; Wickert *et al.*, 2003). Consistently, using a fluorescent lipid and 3D reconstructions of sequential confocal images, Halder and colleagues observed a continuous, membranous tubular network and vesicular structures within the cytoplasm of infected erythrocytes with dots, presumably Maurer's clefts, connected by fine threads originating from the PVM (Halder *et al.*, 2001). Additionally, electron tomography studies showed stalk-like structures connecting one end of Maurer's clefts body to the PVM (Hanssen *et al.*, 2008b). These findings are consistent with the Maurer's clefts originating from the PVM.

However, FRAP-fluorescence recovery after photobleaching using a fluorescent lipid probe and GFP chimeras of Maurer's clefts proteins such as MAHRP1 and REX1 (Ring Exported Protein 1) (Hanssen *et al.*, 2008a; Spielmann *et al.*, 2006b; Spycher *et al.*, 2006) showed that although nascent Maurer's clefts seem to bud from the PVM, they further diffuse in the host cell cytoplasm as distinct, independent entities. Moreover, proteomic and immunofluorescence studies have revealed different sets of proteins residing in the parasitophorous vacuole and Maurer's clefts (Nyalwidhe & Lingelbach, 2006; Vincensini *et al.*, 2005).

4.3 Connectivity of the Maurer's clefts with the host cell membrane

A recent study by Gruring *et al* shows that Maurer's clefts are highly mobile structures in the ring stage parasites and with transition to trophozoite stage, the position of clefts become fixed and with smaller rearrangement in the later stage as clefts predominantly

move to host cell periphery before merozoite formation and egress (Gruring *et al.*, 2011). Consistently, using limited osmotic lysis of infected erythrocytes, Blisnick and colleagues showed that Maurer's clefts are attached to the erythrocyte membrane and sub-membrane skeleton (Blisnick *et al.*, 2000). The binding of Maurer's clefts to the erythrocyte membrane in the late stage parasite partly depends on the interaction of a Maurer's clefts resident protein, PfSBP1 (*P. falciparum* skeleton binding protein1) (Blisnick *et al.*, 2000) with an erythrocyte host peripheral membrane protein, LANCL1 (lantibiotic synthetase component C-like protein) through its carboxy-terminal domain (Blisnick *et al.*, 2005). This interaction is dependent on the phosphorylation status of PfSBP1 which is regulated by a Maurer's cleft protein phosphatase, PfPP1, in the late stage parasite (Blisnick *et al.*, 2006). However, Maurer's clefts are attached to the erythrocyte membrane throughout the intra-erythrocytic development of the parasite (Blisnick *et al.*, 2005). Hence, it is believed that there must be other forms of interaction between Maurer's clefts and the erythrocyte membrane probably involving binding of Maurer's clefts proteins to erythrocyte skeleton proteins such as actin (Etzion & Perkins, 1989) or ankyrin (Atkinson *et al.*, 1988).

Indeed, electron tomography studies revealed that some Maurer's clefts are tethered to the erythrocyte membrane with stalk-like profiles (Hanssen *et al.*, 2008b). High resolution at the tethered region reveals a membrane bilayer tube of a diameter of ~30 nm, with a striated appearance and a more electron dense luminal compartment as compared to the Maurer's clefts lumen (Tilley & Hanssen, 2008). The contact between the tether-like structure and the erythrocyte membrane appears to involve an interaction with the cytoplasmic face of the erythrocyte membrane. In addition, a parasite membrane-associated histidine-rich protein 2 (MAHRP2) has also been identified residing specifically at these stalk extensions (Pachlatko *et al.*, 2010). Importantly, all attempts to date to genetically knock out *mahrp2* have failed, indicating its importance, and that of Maurer's clefts, for the parasite survival. Very new and important data have been recently published, showing that the flattened morphology of Maurer's clefts is likely due to the force generated by actin filaments that polymerize from the Maurer's clefts to domains of the red blood cell sub-membrane skeleton underneath the knobs (Cyrklaff *et al.*, 2011). Vesicle-like structures of ~25 nm in diameter were also observed in the erythrocyte cytoplasm which may be involved in the transport of cargoes between the Maurer's clefts and red cell membrane compartments (Hanssen *et al.*, 2008b). Moreover, the actin filaments attaching Maurer's clefts to the knobs seem to provide support and guidance for the transport of such vesicles from the clefts to the host cell plasma membrane (Cyrklaff *et al.*, 2011).

In conclusion, nascent Maurer's clefts are thought to originate from the parasitophorous vacuole membrane and then mature to form functionally independent compartments tethered to the erythrocyte membrane. These membranous compartments are not physically connected, as there is no bilayer continuum between the compartments at either the protein or lipid level but are connected by vesicles, likely transporting parasite proteins from the Maurer's clefts to the host cell surface (Gruring *et al.*, 2011; Hanssen *et al.*, 2008b; Tilley & Hanssen, 2008).

4.4 Biological roles of Maurer's clefts

Maurer's clefts are described as an extracellular secretory organelle which functions as an intermediate compartment or 'pre-assembly' platform for the sorting and delivery of

parasite-encoded proteins to their final destinations in the host cell (Przyborski, 2008). In addition to permanent resident proteins (Vincensini *et al.*, 2005), Maurer's clefts appeared to house some transient parasite-encoded proteins such as STEVOR (subtelomeric variable open reading frame) (Przyborski *et al.*, 2005), KAHRP (knob-associated histidine rich protein) (Wickham *et al.*, 2001), PfEMP3 (Knuepfer *et al.*, 2005a) and the virulence factor, PfEMP1 (Knuepfer *et al.*, 2005b) en route to their final destinations at the host cell periphery.

Generation of PfSBP1 knock-out parasites showed that this Maurer's clefts resident protein is essential for the export of the PfEMP1 adhesin to the erythrocyte surface; in addition, in these knock-out parasites, the Maurer's clefts morphology was altered and Maurer's clefts were no longer found close to the periphery of the infected erythrocytes (Cooke *et al.*, 2006). Furthermore, over expression, mutagenesis or deletion of other resident or associated Maurer's clefts proteins such as MAHRP1 (Spycher *et al.*, 2008), REX1 (that associates with the edges of Maurer's clefts) (Hanssen *et al.*, 2008a) and PfEMP3 (Waterkeyn *et al.*, 2000) not only alter the morphology, formation and architecture of Maurer's clefts, but also affect the delivery and presentation of the virulence factor PfEMP1 to the erythrocyte surface (Dixon *et al.*, 2011; Maier *et al.*, 2009). All these data demonstrate that the correct architecture and assembly of Maurer's clefts and their connectivity to the host cell membrane are essential for the delivery of PfEMP1 to knobs and the cytoadhesive properties of *P. falciparum*-infected erythrocytes.

Besides playing a role in protein exporting, Maurer's clefts also potentially house chaperones (HSP), metabolic enzymes and proteins involved in signalling pathways (Vincensini *et al.*, 2005). This indicates that Maurer's clefts could be a multifunctional organelle serving as a platform for metabolic pathways and signalling processes such as phospholipids biosynthesis, protein modulation by phosphorylation and dephosphorylation eventually affecting merozoite egress or other biological processes as reviewed in (Lanzer *et al.*, 2006). Hence, it is crucial to have a deeper insight of the organization and compositions of this membrane compartment.

5. Export of parasite proteins to the host cell

Upon merozoites invasion and trophozoite growth, huge erythrocyte remodelling has been made as discussed before for the parasite growth, nutrients acquisition, pathogenesis and immune evasion, by exporting parasite-encoded proteins to the host cell (Figure 3). In doing so, the parasite has to establish its own novel secretory and trafficking system in the host cell that lacks secretory pathways. In higher eukaryotes, the secretion or trafficking of proteins requires a chain of sequential and highly regulated steps that involve budding and fusion of small vesicles. Most proteins destined to be secreted or exported have a stretch of amino-terminal hydrophobic signal sequence for translocation into the endoplasmic reticulum (ER) (von Heijne, 1985) then transit through the Golgi apparatus before exiting from the cell by exocytosis. These series of events are termed as the constitutive secretory pathway. In *P. falciparum*, like in other eukaryotes, secreted proteins undergoing a constitutive secretory pathway have a signal sequence composed of a stretch of about 15 to 20 hydrophobic amino acids from the N-terminal or a recessed N-terminal signal sequence up to 80 amino acids from the N-terminus addressing the protein to the ER (Lingelbach, 1993). Proteins either with the classical or recessed signal sequences are able to follow the "constitutive" or "default" secretory pathway into the parasitophorous vacuole (soluble proteins) or the

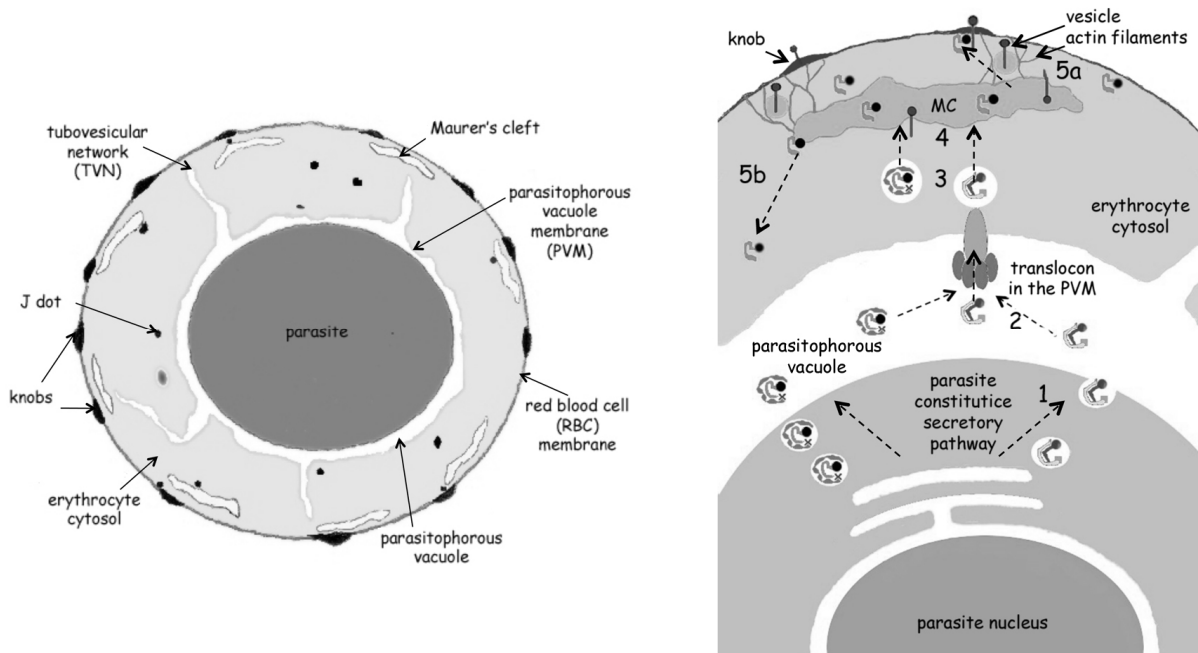


Fig. 3. Scheme of a *P. falciparum*-infected erythrocyte focusing on the host cell major changes (left panel) and a proposed model for export of parasite proteins beyond the confines of the parasite (right panel). The parasite is growing inside the parasitophorous vacuole, the membrane of which constitutes the interface between the parasite and its external environment. Extensions of the parasitophorous vacuole membrane (PVM) form the tubovesicular network (TVN) extending into the host cell cytosol. Various parasite structures are transposed into the red cell cytosol: the Maurer's clefts are flat and elongated membrane vesicles at the host cell periphery and linked to the host cell membrane and sub-membrane skeleton; J dots are likely membrane structures that might traffic some parasite proteins through the erythrocyte cytosol. Complexes of exported parasite proteins interacting with the erythrocyte membrane and sub-membrane skeleton forms protrusions of the red cell membrane, referred to as knobs, that mediate adhesion of the infected erythrocyte to host cells. Parasite proteins exported to the host cell traffic through the parasite constitutive secretory pathway as soluble proteins (1) (membrane proteins are likely interacting with chaperones to maintain them as unfolded and soluble) and are released in the lumen of the parasitophorous vacuole (2). Interacting with chaperones in the parasitophorous vacuole, they are addressed to a translocon in the parasitophorous vacuole membrane (PVM) (2) and released in the host cell cytosol (3). The proteins are further addressed to the Maurer's clefts, as soluble complexes and also possibly associated with J-dots (4). Finally, soluble proteins are sorted from the Maurer's clefts to the red cell cytosol and sub-membrane skeleton (5b) and membrane proteins are trafficked to the red cell plasma membrane (5a), likely by vesicles that fuse with the host cell membrane.

parasite plasma membrane (integral membrane proteins) (Adisa *et al.*, 2003; Tonkin *et al.*, 2006; Wickham *et al.*, 2001). An example is the integral membrane *P. falciparum* exported protein-1 (PfEXP1), which possesses a classical N-terminal signal sequence and is exported beyond the parasite to the PVM (Günther *et al.*, 1991). Another exported protein KHARP, involved in the cytoadherence complex, has a recessed N-terminal signal that contains information both necessary and sufficient for entry into the ER and trafficking to the

parasitophorous vacuole (Wickham *et al.*, 2001). These data indicate that the parasite's translocation machinery is able to recognize both the classical and recessed signal peptides, and in the absence of any additional sorting information, proteins are transported into the parasitophorous vacuole. However, a 34 amino acid sequence in the C-terminal region of eight studied PVM proteins was proposed to be the parasitophorous targeting motif (Eksi & Williamson, 2011). On the other hand, additional information is required for most proteins, which are exported beyond the confines of the parasite across the parasitophorous vacuole to the erythrocyte cytosol and surface.

The unusual nature of export process of *Plasmodium* is further signified by the discovery of a novel pentameric amino acid sequence motif that directs the export of parasite encoded proteins beyond the parasitophorous vacuole. This conserved motif (R/KxI/LxE/Q/D) is referred to as *Plasmodium* Export Element (PEXEL) (Marti *et al.*, 2004) or alternatively as Vacuolar Targeting Signal (VTS) (Hiller *et al.*, 2004), which are identified by different algorithms with slightly different specificities but recognizing the same core sequence (van Ooij *et al.*, 2008). Interestingly, a similar Host Cell Targeting motif (HCTM) is also detected in the Irish potato famine pathogen *Phytophthora infestans* for delivering virulence gene products into plant cells (Bhattacharjee *et al.*, 2006). This has provided the first evidence that eukaryotic microbes share equivalent targeting signals and thus possible conserved mechanisms to access host cells (Halder *et al.*, 2006). The PEXEL motif is located about 20-40 amino acids downstream from the signal sequence and is typically encoded in close proximity to the start of exon 2 in a two-exon gene (Charpian & Przyborski, 2008). The discovery of this motif, allowed the *in silico* prediction of the exported proteins of *P. falciparum* and other *Plasmodium* species. Using different algorithms, the *P. falciparum* exportome was predicted to contain more than 300 proteins (Hiller *et al.*, 2004; Marti *et al.*, 2004; Sargeant *et al.*, 2006). Many of these proteins have one or two predicted trans-membrane domains (Sargeant *et al.*, 2006; van Ooij *et al.*, 2008) indicating that the parasite transport machinery can export both soluble and trans-membrane proteins. In addition, the amino acids surrounding the motif are important for the correct targeting or trafficking to the host cell as demonstrated by (Przyborski *et al.*, 2005) for the efficient trafficking process of STEVOR.

Further dissecting this PEXEL motif, recent studies provided evidence of N-terminal processing of this motif as shown for PfEMP2, PfHRP2 (Chang *et al.*, 2008), PfKAHRP and GBP130 (Glycophorin Binding Protein) (Boddey *et al.*, 2009), where this motif is recognized by a novel ER peptidase which cleaves on the C-terminal side of the Leucine residue in the PEXEL motif. Plasmeprin V is proved to be the ER-resident peptidase responsible for this cleavage (Boddey *et al.*, 2010; Russo *et al.*, 2010). The new N-terminus is then further acetylated in the parasite ER in a PEXEL independent process (Boddey *et al.*, 2009; Chang *et al.*, 2008). The processed protein should then present a motif that is recognized by a specific transporter in the parasitophorous vacuole membrane that helps translocating the protein across the PVM to the host cytosol. Indeed, a *Plasmodium* translocon of exported proteins (PTEx) located in the PVM has been identified in *P. falciparum* (de Koning-Ward *et al.*, 2009). This translocon is ATP-powered and comprises heat shock protein 101, which belongs to a super family commonly associated with protein translocons, a novel protein termed PTEx150 (PF14_0344) and a known parasite protein, exported protein 2 (EXP2), which is suggested to be a potential channel since it is the membrane-associated component of the

core PTEX complex (de Koning-Ward *et al.*, 2009). PfEXP2 lacks a typical hydrophobic trans-membrane domain but was proved to be membrane-associated via an amphipathic helix located at the N-terminal part of the protein (Fischer *et al.*, 1998). It has been proposed that, like bacterial pore forming proteins to which it shows some structural similarities, PfEXP2 might insert into the PVM by oligomerization (Haase & de Koning-Ward, 2010). N-acetylation may help the PVM translocon to differentiate between proteins to be exported beyond the PVM and those that should reside in the parasitophorous vacuole. In addition, protein unfolding maintained with the help of chaperones is an essential requirement for transport across the PVM (Gehde *et al.*, 2009). Chaperones and proteases are the most abundant proteins in the vacuole, suggesting an important role of the vacuole both in protein folding and processing (Nyalwidhe & Lingelbach, 2006).

Chimeric proteins with (Wickham *et al.*, 2001) or without (Spycher *et al.*, 2006) PEXEL motif located near the parasite periphery have been reported to localize to structures with the appearance of a necklace of beads that are resistant to recovery after photobleaching. These data suggest the presence of sub-compartments within the PVM. In addition, PfEXP1 and ETRAMP locating at the PVM define separate arrays demonstrating that the protein distribution in the PVM is non-random, hence reinforcing the idea of the presence of sub-compartments within the PVM (Adisa *et al.*, 2003; Spielmann *et al.*, 2006a). Such sub-compartments are proposed to house the PTEX translocon (Boddey *et al.*, 2009; de Koning-Ward *et al.*, 2009).

Exceptionally, the PEXEL motif is not sufficient to export a parasite protein into the host cell as illustrated by RIFIN proteins: members of the B-type subfamily of RIFINs are exported to the Maurer's clefts while subfamily A-type RIFINs are retained within the parasite despite having the PEXEL motif (Petter *et al.*, 2007). This observation highlights the role of additional motifs or protein-protein interactions for efficient export that might be even more important than the PEXEL motif since an increasing number of parasite proteins that lack such an export motif are reported. These proteins are termed as PEXEL negative proteins or PNEPs [(Spielmann *et al.*, 2006b) and reviewed in (Spielmann & Gilberger, 2010)]. Some of the PNEPs including PfSBP1 (Saridaki *et al.*, 2009), PfMAHRP1 (Spycher *et al.*, 2008) and PfREX-1 (Spielmann *et al.*, 2006b) are known to be exported to the Maurer's clefts. The trans-membrane domain of PfSBP1 was demonstrated to address the protein to the parasite ER and constitutive secretory pathway. One of the two characterized N-terminal domains of PfSBP1 with high negative net charge and acting independently is necessary for the protein export beyond the parasite to Maurer's clefts (Saridaki *et al.*, 2009). For PfMAHRP1, the second half of the N-terminal part of the protein and the trans-membrane domain contain the essential signal for trafficking to Maurer's clefts (Spycher *et al.*, 2006). As for PfREX-1, a hydrophobic stretch and additional 10 amino acid towards the C-terminal are important for the protein export (Dixon *et al.*, 2008). The PfSURFIN_{4.2} protein was shown to localize at the Maurer's clefts and the infected erythrocyte plasma membrane using immuno-electron microscopy (Winter *et al.*, 2005), and found to be trafficked to the host cell as a PNEP. PfSURFIN_{4.2} protein export to the host cell does not depend on any of its two non consensus PEXEL-like motifs nor on its extracellular domain but requires its predicted trans-membrane domain (Alexandre *et al.*, 2011). Interestingly, PfSURFIN_{4.2} was reported to accumulate in the parasitophorous vacuole in late schizonts, thus suggesting stage-dependent differential localization (Winter *et al.*, 2005). Taken together, these studies showed that no obvious export motif is found among and shared by PNEPs but proved the importance of an

hydrophobic trans-membrane domain, likely addressing PNEPs to the parasite ER, and that of protein-protein interactions for their delivery beyond the confines of the parasite. Whether PNEPs indirectly use the PTEX translocon or an alternative export pathway calls for more investigations.

To date, there are many proposed models of protein trafficking pathways across the PVM to the erythrocyte cytosol and surface, based on the studies of different parasite proteins which has broaden our knowledge of the presence of multiple exporting routes. The most popular model of protein export across the PVM is that unfolded proteins are secreted into the parasitophorous vacuole, and translocate through a channel or translocon (PTEX) into the host cytosol as discussed above. Ultrastructural studies showing strings of vesicles budding off from the PVM have provided evidence of vesicle trafficking in the infected erythrocyte cytosol (Trelka *et al.*, 2000). PfEMP1 and PfEMP3 were found to be associated with these vesicles, and proposed to be delivered to the erythrocyte surface in the mode of vesicles (Trelka *et al.*, 2000). In addition, homologues of two components of the classical vesicle-mediated trafficking machinery COPII, PfSar1p and PfSec31p, were reported to be exported to the erythrocyte cytosol, suggesting a vesicle-mediated trafficking pathway for proteins across the erythrocyte cytoplasm (Adisa *et al.*, 2001; Adisa *et al.*, 2002). However, this model has been recently challenged because, even in the presence of slowly hydrolysable GTP analogues blocking vesicular trafficking, PfEMP1 was still properly trafficked to the erythrocyte membrane (Frankland *et al.*, 2006). Moreover, the localization of the COPII proteins has been later redefined inside the parasite cytoplasm (Adisa *et al.*, 2007). Furthermore, PfEMP1 is transported as a soluble chaperoned complex in the erythrocyte cytosol, transiently inserts into the Maurer's clefts membrane and finally inserts into the erythrocyte membrane (Papakrivos *et al.*, 2005). This has revealed another model of non-vesicular mode of protein export where proteins may transport as soluble complexes in the erythrocyte cytosol and then interact transiently with Maurer's clefts before reaching the erythrocyte membrane skeleton (Knuepfer *et al.*, 2005a). Similarly, PfREX1 is exported across the PVM to the host cell cytosol as a soluble form and inserts to Maurer's clefts *via* a putative coiled-coil motif (Dixon *et al.*, 2008). Differently, PfMAHRP1 is trafficked to the Maurer's clefts in a membrane-associated manner budding from the PVM (Spycher *et al.*, 2006), adding to the evidence that nascent Maurer's clefts might be connected to or bud from the PVM as previously discussed.

To further elucidate the mechanisms of protein trafficking, Hanssen and collaborators have applied immunoelectron tomography combined with serial sectioning and immunogold labelling to explore the topography of infected erythrocytes (Hanssen *et al.*, 2010). They proposed that the exported secretory system of *P. falciparum* comprises a series of modular units: TVN, Maurer's clefts, and two different populations of vesicles of 25 and 80 nm in diameter in the erythrocyte cytosol, suggesting the presence of a vesicular-mediated trafficking pathway for the delivery of cargo between compartments to different destinations in the host cell (Hanssen *et al.*, 2010). Recently, other extra-parasitic structures named 'J-dots' and containing the exported parasite Hsp40 co-chaperone, were identified in the infected erythrocyte cytosol and proposed to traffic parasite proteins to the host cell (Kölzer *et al.*, 2010). However, all parasite proteins identified so far as exported to the host cell are transiently associated with the Maurer's clefts. Since Maurer's clefts are physically tethered to the erythrocyte membrane, Hanssen and collaborators have proposed that proteins traffic from the Maurer's clefts to the erythrocyte membrane *via* the membranous

tubular structure tethering the clefts to the host cell membrane (Hanssen *et al.*, 2010). Alternatively, transport vesicles have been shown to be attached to the actin filaments that connect the Maurer's clefts to the host cell membrane and might sustain the transport of proteins between these two compartments (Cyrklaff *et al.*, 2011).

6. Lipids remodelling: Implications of lipid rafts (DRMs) in human malaria

Despite identifying the roles and biogenesis of specific extracellular compartments of the parasite and the discovery of the protein exporting PEXEL motif with different models of trafficking pathways proposed, the contribution of lipids in these cellular processes is poorly understood even though the exported proteins have to bypass several membrane barriers to reach their final destination. Upon merozoite invasion, there is a change in the lipid and protein compositions of the infected erythrocyte membrane indicating that the parasite also remodels micro-domains of its host cell membrane known as lipid rafts and a lipid raft-based biogenesis of the parasitophorous vacuole membrane has been proposed. In addition, lipid raft-based processes and interactions of both host and parasite origin might be crucial to maintain the stability of the vacuolar environment for the parasite growth and pathogenesis [reviewed in (Murphy *et al.*, 2006)].

Lipid rafts also serve as a stage for protein assemblies, sorting and trafficking through endocytic and secretory pathways in other cell types [reviewed in (Hanzal-Bayer & Hancock, 2007)]. Do DRMs have any contributions to *P. falciparum* protein trafficking pathways in infected erythrocytes? Tamez and colleagues have described a vesicle-like membrane compartment in the infected erythrocyte cytosol, which might be implicated in the import of lipids from the erythrocyte membrane to the TVN (Tamez *et al.*, 2008). Moreover, the binding of the parasite Hsp40 co-chaperone to "J-dots", proposed to be involved in protein trafficking through the erythrocyte cytosol, was shown to be cholesterol dependent (Külzer *et al.*, 2010). Furthermore, the presentation of the parasite virulence protein PfEMP1 on the erythrocyte surface involves the final insertion of the protein into cholesterol-rich domains of the erythrocyte plasma membrane (Frankland *et al.*, 2006) and with more delivery in the presence of serum lipoproteins (Frankland *et al.*, 2007). Whether all parasite proteins exported to the host cell surface are delivered to lipid rafts needs to be further investigated.

In conclusion, elucidating and characterizing the functional roles of cholesterol rich DRMs during the intra-erythrocytic development of the *P. falciparum* parasite might shed new light on protein trafficking or host cell remodelling processes.

7. Merozoite egress from the red cell: A split second event likely depending on very early changes to the red blood cell membrane

The release of infectious merozoites from the host cell requires the opening of the parasitophorous and red cell membranes. Dvorak and collaborators have first observed that the swelling of the infected erythrocyte precedes the egress of *Plasmodium falciparum* merozoites by a few minutes (Dvorak *et al.*, 1975). In addition, the use of amphiphiles, osmotic stress and protease inhibitors strongly suggested that merozoite release is pressure driven (Glushakova *et al.*, 2009; Glushakova *et al.*, 2005). Shortly before merozoite egress, the intracellular parasites seem to move more freely while the red cell membrane is still intact

(Abkarian *et al.*, 2011), comforting previous studies providing evidence that, when the merozoites are close to egress, the PVM enlarges and ruptures before the erythrocyte membrane (Wickham *et al.*, 2003). What drives a sudden increase in the osmotic pressure? A premature release of immature merozoites has been recently described which results from the inhibition of RNA degradation and is preceded by swelling of the infected erythrocyte (Balu *et al.*, 2011). In addition, parasite proteases specifically active just prior to merozoite release could also participate in the increased osmolarity (Koussis *et al.*, 2009). Noteworthy, proteases of both parasite and host origin have likely numerous roles in merozoite egress and might also participate in both the rupture of the PVM and the subsequent opening of the erythrocyte membrane (Arastu-Kapur *et al.*, 2008; Chandramohanadas *et al.*, 2009; Yeoh *et al.*, 2007).

Indeed, although first considered as an explosive event, merozoite egress from the red blood cell has been shown recently to occur through the opening and stabilization of an osmotic pore in the host cell membrane allowing the release of a limited number of merozoites (Abkarian *et al.*, 2011). The pore opening is followed by the curling and buckling of the erythrocyte membrane, and this results in the wide-angular dispersion of the remaining merozoites. These events happen when a critical radius of the osmotic pore is reached. Abkarian *et al.* 2011 hypothesized that this instability is biologically relevant as it disperses the merozoites and contributes to separate them efficiently from the infected cell membrane. Indeed, abortive egress events have been observed with a stop of curling and no buckling, resulting in the merozoites remaining stuck together inside the open erythrocyte and thus unable to further invade new red blood cells (Abkarian *et al.*, 2011). Noteworthy, these data have been obtained with infected erythrocytes in suspension and it is important to determine whether merozoites release proceeds through similar steps *in vivo*, when red cells with mature parasites are sequestered in the microvasculature, adhering to endothelial cells. Observations of infected erythrocytes adhered to a glass substrate shed some light on this process: over 5 merozoites were sequentially released through a pore of similar radius (1 μm) and with higher velocity as compared to non adhering cells, before curling occurred. The membrane was then projected backwards, thereby releasing merozoites but without actually pushing them forward. In brief, while similar steps are involved, the resulting dispersion of the merozoites looks different. These results suggest that adhesion maintains a membrane tension high enough to produce the overpressure driving more merozoites out of the host cell. Considering that *P. falciparum* infected erythrocytes are also able to adhere to non-infected erythrocytes, the merozoites would be released appropriately to re-invade *in vivo* efficiently.

The curling and buckling of the infected erythrocyte membrane can originate from an additional elastic energy due to an asymmetry between the membrane leaflets (Abkarian *et al.*, 2011). A nice illustration of this effect is the curling of a gift ribbon after one slides it between the thumb and a scissor blade, thus creating an excess area of the outer leaflet (Klaes *et al.*, 2007). In *P. falciparum*- infected erythrocytes, this asymmetry between the two membrane leaflets could originate from a lipid excess in the inner leaflet caused by a lipid release of parasite origin, a modification of the mechanical properties of the red cell membrane through changes of the cytoskeleton/membrane interactions [reviewed in (An & Mohandas, 2010)] and/or interactions of the erythrocyte membrane with the Maurer's clefts.

As described before, parasite-induced changes at the red blood cell membrane affecting its stability occur as early as parasite entry and very early intra-erythrocytic growth. Moreover, phosphorylation of host peripheral proteins increases upon parasite growth and might modulate the bio-physical properties of the red cell membrane throughout the parasite development (Pantaleo *et al.*, 2010). One might thus consider that on one hand the parasite weakens its host cell membrane and on the other hand it stabilizes it by exporting proteins to the red cell sub-membrane skeleton and recruiting host proteins to or from the sub-membrane skeleton.

However, the ability to curl and buckle has also been proposed to be an intrinsic property of the erythrocyte membrane when the cell is exposed to certain osmotic stress (Lew, 2011) although with marked kinetic differences as compared to the infected erythrocyte. Whether the parasite explores a property of its host cell and at what extent the changes of the red cell membrane and sub-membrane skeleton induced by the parasite are essential for efficient merozoite release need further investigations.

8. Concluding remarks

As described in this chapter, Apicomplexan parasites widely transform the parasitophorous vacuole in which they grow and multiply and which constitutes the interface between the parasite and its extracellular environment. Changes of its closed environment, the red blood cell cytoplasm and plasma membrane, induced by the life-threatening human malaria parasite *Plasmodium falciparum* have been extensively studied because these changes are crucial for the parasite development and some, referred to as knobs, are specific for this species and central to the pathogenesis of severe malaria. In the last decade, the set up of *P. falciparum* genetic engineering and the spectacular advances of imaging technologies, have considerably highlighted our knowledge of the red cell remodelling by the parasite, the processes involved and their importance for the parasite survival.

Upon intra-erythrocytic parasite growth, new permeation pathways in the red cell membrane and extensions of the parasitophorous vacuole membrane in the host cell cytosol, named the tubovesicular network, participate in the import of nutrients from the extracellular milieu. Other membrane structures transposed by the parasite in the cytoplasm of its host cell, referred to as Maurer's clefts, and proposed to generate from the parasitophorous vacuole membrane, are central to the transport of parasite proteins to the red blood cell. They tightly interact with the host cell membrane even upon merozoite release. This interaction together with exported parasite proteins interacting with the host cell sub-membrane skeleton might prevent the premature rupture of the red cell membrane and consequent release of immature merozoites. Maintaining the integrity of the red cell membrane upon its growth is likely crucial for the parasite because it has weakened its host cell membrane by altering the cohesion between the plasma membrane and sub-membrane skeleton *via* the phosphorylation and the recruitment of host cell membrane and skeletal proteins. On the other hand, one can consider that the parasite has prepared its host cell membrane not only for entry but also for egress because reversing the parasite-induced modifications, for example by the activation of phosphatases, would highly facilitate the rupture of the red cell plasma membrane.

The merozoite release, following the engulfment of the infected erythrocyte, relies on a unique site of opening allowing the egress of the first one or two merozoites; the release of the remaining merozoites results from the curling and eversion of the red blood cell membrane. Importantly, the same sequence of events has been observed whether the infected erythrocytes were in suspension or adhering to the substrate (which is the usual status of *P. falciparum* infected erythrocytes because of cytoadherence to the micro-vessel endothelium and to non-infected erythrocytes). The physical parameters of curling and eversion of the red cell membrane emphasized once more the importance of parasite-induced changes to the host cell membrane.

Red blood cell remodelling by the malaria parasite necessitates both efficient export of parasite proteins to the host cell and extensive membrane synthesis. These processes, together with the parasite enzymatic activities, such as proteases, protein kinases and phosphatases, which are crucial for the intra-cellular survival of the parasite and evasion from splenic clearance and host immune response, deserve precise characterization because they are Achilles heels that could be targeted by specific drugs or antibodies.

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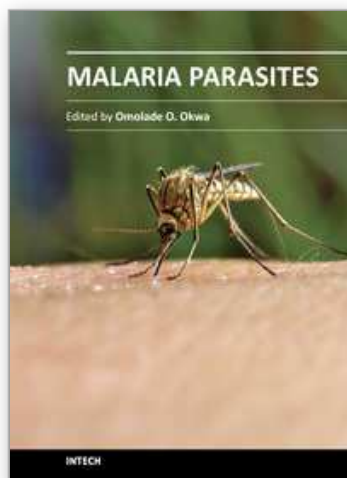
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Malaria is a global disease in the world today but most common in the poorest countries of the world, with 90% of deaths occurring in sub-Saharan Africa. This book provides information on global efforts made by scientist which cuts across the continents of the world. Concerted efforts such as symbiont based malaria control; new applications in avian malaria studies; development of humanized mice to study *P.falciparum* (the most virulent species of malaria parasite); and current issues in laboratory diagnosis will support the prompt treatment of malaria. Research is ultimately gaining more grounds in the quest to provide vaccine for the prevention of malaria. The book features research aimed to bring a lasting solution to the malaria problem and what we should be doing now to face malaria, which is definitely useful for health policies in the twenty first century.

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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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