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Mini Review

Molecular mechanisms of host cell egress by malaria parasites

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ABSTRACT

Egress is a crucial step for malaria parasites to progress from one host cell to another. The rapid transition between host cells is mediated by the invasive merozoite stages. Merozoite egress from the enveloping cell includes the rupture of two membranes, the membrane of the parasitophorous vacuole and the host cell membrane. Egress from the host cell is also of importance for the intraerythrocytic gametocytes in order to undergo gametogenesis following their transmission to the mosquito during the blood meal. An increasing number of studies have aimed to identify the molecules involved in host cell egress by malaria parasites and decipher the sequence of membrane rupture. Recent work has acknowledged the crucial roles of plasmodial and host-derived proteases in membrane rupture and has indicated the involvement of secretory vesicles in priming the enveloping membranes for egress. This review highlights recent insight into the mechanisms of host cell egress by *Plasmodium* parasites. We will discuss the mode of egress of intrahepatic and intraerythrocytic parasites and their measures to evade the host immune system during this process.

Introduction

Plasmodium parasites are the causative agents of the tropical disease malaria, which causes 216 million new cases and approximately 655,000 deaths every year (WHO World Malaria Report, 2011). The life cycle of *Plasmodium* alternates between the human host and the Anopheline mosquito vector, and during life cycle progression, three major stages can be distinguished, i.e. the liver stages, the blood stages, and the mosquito-specific stages.

Plasmodium ssp. are obligate intracellular parasites that reside within a membrane-bound vacuole for the most part of their life cycle. Cell invasion occurs by active penetration of the host cell (HC), i.e. the hepatocyte or the erythrocyte, with the formation of a parasitophorous vacuole (PV). The motile HC-invading stages, the sporozoites and merozoites, promote an invagination of the HC plasma membrane (HCM) with the formation of a junction between the two cells. During invasion, HCM-derived integral membrane proteins are excluded from the PV membrane (PVM), while parasite-derived proteins and lipids are inserted, resulting in major modifications of this membrane (reviewed by Charpian and Przyborski, 2008; Zuccala and Baum, 2011).

As for all intracellular-living pathogens, subcellular compartmentalization is a feature of the malaria parasite for avoiding the host immune system. Living inside an HC protects the parasite from host immune defense mechanisms and also provides the parasite a ready source of nutrients. Consequently, malaria parasites aim to minimize the time spent outside HCs. The rapid transition between HCs is mediated by the short-lived merozoites, and tools used to successfully mediate this transition include an actin/myosin-based gliding motility and specialized apical secretory organelles important for HC invasion, particularly the micronemes and rhoptries (reviewed in Baum et al., 2008).

Another type of transition is mediated by gametocytes, sexual precursor cells formed in the human blood when the parasite prepares to switch from the human to the mosquito. The intraerythrocytic gametocytes are taken up by the female *Anopheles* mosquito during a blood meal. In the mosquito midgut, the gametocytes egress from the HC and transform into extracellular gametes, thereby initiating sexual reproduction.

The escape from the HC is crucial for the malaria parasite to progress though its life cycle and to promote propagation. Parasite egress from the HC follows a fixed program and involves the sequential rupture of two membranes, the PVM and the HCM. Until recently, the order of membrane rupture had been under debate (reviewed in Blackman, 2008). However, a number of recent publications provided increasing evidence for an inside-out egress of the malaria parasite from the HC, during which the break-down of the PVM precedes rupture of the HCM (Glushakova et al., 2010; Chandramohanadas et al., 2011; Graewe et al., 2011; Sologub et al., 2011).

This review highlights recent findings on the egress of the three different intracellular parasite stages, i.e. the liver stages, the blood stages, and the gametocytes, from the respective HCs. We

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investigate the mechanisms and molecules involved in HC egress and discuss the tricks the parasites employ to evade the host immune system during this process.

Host cell egress by the intrahepatic stages

The mammalian liver is the primary site of infection and replication for the malaria parasite *Plasmodium*. The molecular mechanisms underlying the egress of *Plasmodium* from the hepatocyte are mostly investigated in rodent malaria models such as *P. berghei* due to the easier accessibility of sporozoites, but the mechanisms are very likely to be transferable to human malaria parasites.

Productive invasion of *Plasmodium* sporozoites is followed by their rapid multiplication into approximately 20,000 first-generation merozoites, each of which is able to initiate the erythrocytic cycle accompanied with malaria disease (reviewed in Prudêncio et al., 2006). After successful liver cell invasion the parasite resides for several days (2–3 days in rodent malaria parasites; 5–10 days in human malaria parasites) in this organ, where it differentiates into a spherical schizont. The complete maturation of the merozoites is required for the initiation of parasite egress, which starts with the rupture of the PVM (Graewe et al., 2011). HC death occurs immediately after disintegration of the PVM, accompanied by the release of merozoites into the HC cytoplasm, and is characterized by cell detachment from the liver tissue as well as condensation of the nucleus and loss of the mitochondrial membrane potential (Sturm et al., 2006, 2009). This type of organized cell death shows distinct differences to apoptosis or necrosis, because the infected hepatocyte maintains an intact cell membrane, as demonstrated by the presence of the phagocytosis marker molecule phosphatidyl serine on the cytosolic side of the HCM (Verhoven et al., 1995). Furthermore, the hepatocyte DNA remains unprocessed (Sturm et al., 2006) and caspases are not involved in the HC death (van de Sand et al., 2005). During PVM rupture, calcium is released from internal stores of the dying host hepatocyte. Interestingly, the merozoites counteract the increase of host-derived calcium by actively accumulating the calcium in order to prevent calcium-induced apoptosis of the HC (Sturm et al., 2006). The properties of *Plasmodium*-induced hepatocyte death exhibit striking similarities with a mechanism of programmed cell death induced by salmonella, called pyroptosis (reviewed in Labbe and Saleh, 2008).

The exact molecular processes taking place during parasite egress from the hepatocyte are not known so far, but it has been shown that PVM breakdown, degradation of mitochondria, cell detachment and finally merosome formation are sensitive to the cysteine protease inhibitor E64 (Sturm et al., 2006). Cysteine proteases of the serine repeat antigen (SERA) family, which comprises nine members in *P. falciparum* and five proteins in *P. berghei* (Arisue et al., 2007) are being analyzed for their potential participation in membrane disintegration during liver stage egress. For four of the *P. berghei* SERAs, an up-regulation of protein expression in the late liver stages has been reported (Schmidt-Christensen et al., 2008; Tarun et al., 2008). *Pb*SERA3 can be detected in the PV, and the protein is released into the hepatic cytoplasm following rupture of the PVM (Schmidt-Christensen et al., 2008). Another plasmodial protein involved in HC egress by liver stage parasites, termed LISP1 (liver-specific protein 1), was reported to locate to the PVM of the late liver stages (Ishino et al., 2009). Intrahepatic parasites deficient in LISP1 develop into infective merozoites, which are unable to rupture the PVM and thus remain trapped inside the HC. Other than a signal peptide, no functional domain has been predicted for LISP1, thus the molecular activity of the protein is still not known. It has been proposed, though, that LISP1 is either involved in further SERA processing or that it represents a receptor for proteases during membrane rupture (Ishino et al., 2009).

The actual egress of the merozoites from the HC is initiated by the detachment of the hepatocyte. Mediated by a budding process, the parasites are released into the sinusoidal bloodstream enveloped in HCM-derived vesicles, the so called merosomes, which contain hundreds to thousands of merozoites (Sturm et al., 2006; Baer et al., 2007; Graewe et al., 2011). This process was analyzed in two rodent malaria models, *P. berghei* and *P. yoelii*, and it was proposed that budding of merosomes might occur in a paracellular way, i.e. the merosomes exit the liver by squeezing between two adjacent cells of the fenestrated endothelium of the liver sinusoid. After entering the blood vessels, the merosomes travel into the pulmonary microvasculature. Because the merosomes travel in the blood flow with a lower speed than the erythrocytes, an interaction between the endothelial layer and the merosomal surfaces was postulated (Sturm et al., 2006; Baer et al., 2007). After the merosomes reach the pulmonary capillary system, they accumulate here and eject the infective merozoites into the capillaries by a yet unknown mechanism (Baer et al., 2007). The release of merozoites from the liver in the form of merosomes is probably a measure of the parasite to circumnavigate the Kupffer cells, sessile macrophages of the liver, which colonize the endothelial lining. The lung microvasculature, on the other hand, exhibits a lower macrophage density than the liver capillaries, and the reduced blood velocity and thus reduced shear forces might enhance the ability of the merozoites to invade erythrocytes (Baer et al., 2007).

The combined data on HC egress by intrahepatic parasites point at a release of egress-specific molecules into the PV lumen, where they accumulate and co-localize with the PVM, while death of the liver HC is initiated by the parasite (Fig. 1A). After merozoite formation, the PVM ruptures and the HC detaches from the liver tissue. HCM-based merozoite-containing merosomes are budding into the capillaries and travel to the pulmonary microvasculature, where the merozoites are then released from the vesicles into the blood stream. While the signals and mechanisms underlying the merozoite release from the merosomes are not yet known, it is possible that parasite and/or HC-derived proteases slowly degrade the cytoskeletal elements of the merosomal membrane until it is too instable to stand mechanical influences such as the pressure in the microvasculature of the lung.

Merozoite egress during blood stage replication

The mechanisms of egress have best been studied in the erythrocytic stages of the human malaria parasite *P. falciparum* because of the easy cultivation and accessibility of these stages. Originally, the egress of merozoites from the erythrocyte was investigated by treating blood stage parasites with specific protease inhibitors, particularly E64. These early studies resulted in contradictory data and reported that the inhibitor blocked either degradation of the PVM (Salmon et al., 2001; Wickham et al., 2003; Soni et al., 2005) or the erythrocyte HCM (Glushakova et al., 2009).

A new biophysics analysis by Chandramohanadas et al. (2011) reported that the membrane-permeable inhibitor E64d and the chelator EGTA-AM inhibit the rupture and increase the stiffness of the erythrocyte HCM. However, the inhibitors permitted PVM rupture, since soluble GFP was observed leaking from the parasite to the RBC cytoplasm of inhibitor-treated schizonts. Leakage of soluble GFP from the cytoplasm of a transient GFP-expressing parasite line into the RBC cytosol during egress was previously observed by Wickham et al. (2003) as well, and these combined data strongly support the idea of an inside-out egress of merozoites, in which the PVM ruptures minutes before the breakdown of the HCM.