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Previews

To Migrate or to Invade: Those Are the Options

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Plasmodium sporozoites penetrate and migrate through multiple cells in the host before productively invading a hepatocyte and forming a parasitophorous vacuole. In this issue of *Cell Host & Microbe*, Coppi and colleagues show that sporozoite interaction with the highly sulfated heparan sulfate proteoglycans on liver cells induces proteolytic cleavage of the major sporozoite surface molecule. They conclude that this interaction is the primary trigger that activates sporozoites for productive invasion.

The liver stage is the first, obligatory step of any natural malaria infection. *Plasmodium* sporozoites reach the liver after being deposited into the skin of their vertebrate host through the bite of an infected female *Anopheles* mosquito (Figure 1). After injection into dermal tissue, sporozoites use gliding motility to travel through the skin and into dermal vessels (Prudencio et al., 2006). Once inside the circulatory system, sporozoites rapidly reach the liver sinusoids.

The marked tropism displayed by sporozoites toward the hepatocyte suggests the occurrence of specific interactions between *Plasmodium* surface protein(s) and host molecule(s). The highly sulfated heparan sulfate proteoglycans (HSPGs) on liver cells were shown to play a crucial role in these processes through their interactions with the circumsporozoite protein (CSP), the sporozoite's major surface protein (Pinzon-Ortiz et al., 2001; Sinnis and Coppi, 2007). HSPGs are a family of glycoproteins present on the cell surfaces and in the extracellular matrix of most mammalian cells where they function as coreceptors for growth factors and matrix proteins. In this issue of *Cell Host & Microbe*, Coppi et al. (2007) reveal a previously unknown role for these liver HSPGs during *Plasmodium* infection.

During their journey to the liver, sporozoites likely traverse several cell types, namely the dermal cells in the vicinity of their injection site, endothelial cells that line blood vessels, and Kupffer cells in the liver sinusoids (Figure 1). In addition, sporozoites traverse several hepatocytes in the liver before productively invading a final one, with formation of a parasitophorous vacuole (Mota et al., 2001). The molecular mechanisms that govern the choice between the sporozoites migratory behavior and "productive invasion" are still not fully understood. Now, Coppi et al. (2007) show that the degree of sulfation of HSPGs constitutes a molecular signal for the switch between these parasite behaviors. They demonstrate that processing of CSP by proteolytic cleavage, which is known to occur when sporozoites contact hepatocytes (Coppi et al., 2005), is induced by highly sulfated HSPGs. Furthermore, they show that the invasion process involves signaling by a calcium-dependent protein kinase, probably acting in concert with other signaling events. Together, the findings of Coppi et al. constitute a valuable contribution to the understanding of the molecular events that take place during malaria liver stage infection.

Coppi and colleagues show that inhibition of productive invasion by treatment with an inhibitor of CSP processing leads to a significantly extended sporozoite migration through cells. This observation not only confirms the requirement of CSP cleavage for invasion with parasitophorous vacuole formation but also indicates that sporozoites continue to migrate in the absence of a molecular trigger for productive invasion. Indeed, an inverse correlation was found between sporozoite migration and both CSP proteolytic cleavage and invasion, whereas a positive correlation was shown to exist between the latter two events. The link found between the sulfation degree of HSPGs, productive invasion, and CSP processing is demonstrated in a series of elegantly designed experiments. The authors show that migration is significantly increased by chemical inhibition of sulfation or by incubation of sporozoites with cells that bear a specific deficiency in the degree of sulfation of their heparan sulfate chains, thus convincingly establishing a link between sulfation and productive invasion. Since the latter requires the processing of CSP, it would be natural to assume that CSP cleavage might be dependent upon the sulfation degree of HSPGs. Coppi et al. (2007) firmly establish the connection between these two events by demonstrating that, not only soluble heparin but, most importantly, highly sulfated HSPGs on the hepatocyte surface, induce the proteolytic cleavage of CSP and, hence, modulate sporozoite infectivity.

When sporozoites interact with the highly sulfated liver HSPGs, it can be assumed that a chain of signaling events follows that leads to CSP cleavage and productive invasion. This assumption is supported by the observation of Coppi et al. (2007) that treatment of sporozoites with a protein kinase (PK) inhibitor leads to an increase in migration and to the concomitant inhibition of CSP processing and invasion. In their quest for the PK(s) likely to be involved in this process, the authors identified a *Plasmodium* gene, which they named *CDPK-6*, whose product plays a crucial role in sporozoite

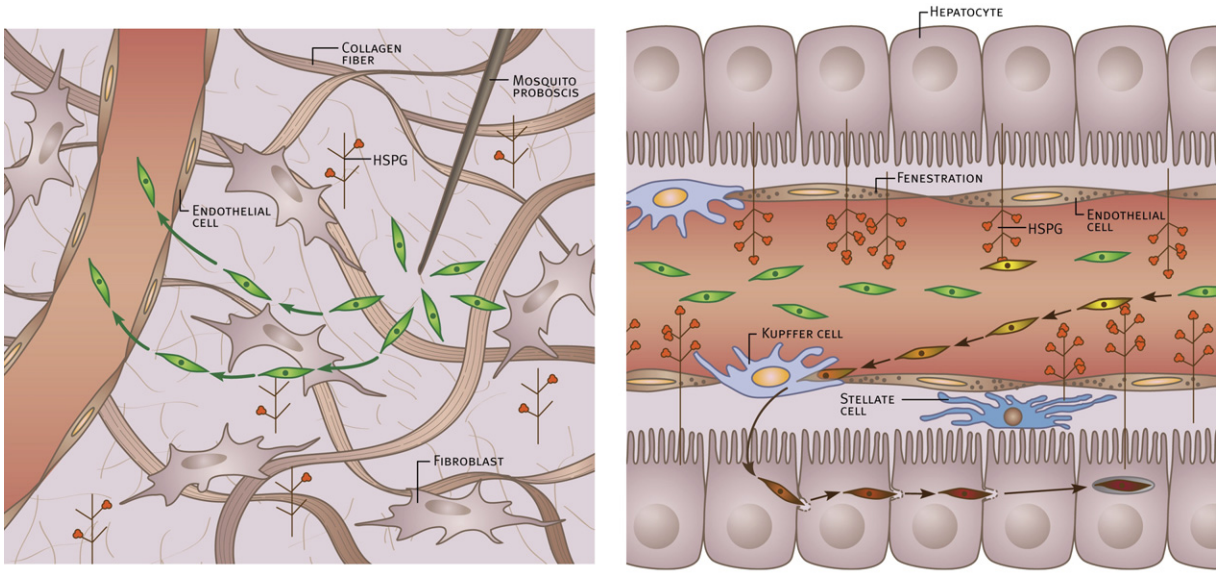

Figure 1. *Plasmodium* Sporozoites Entering the Dermis of the Vertebrate Host (Left Panel) and Infecting the Liver (Right Panel)
Plasmodium sporozoites (green) are deposited under the skin of the vertebrate host through the bite of an infected female *Anopheles* mosquito. After injection into the skin, the sporozoites move through the dermis, which does not express highly supfated HSPGs, until they contact a blood vessel (red) and move into the circulatory system, which allows them to travel to the liver. Once the sporozoites reach the liver sinusoids, they glide over the endothelium and interact with highly sulfated HSPGs, which provide a signal to initiate a program that leads to productive invasion. They then cross the sinusoidal layer, possibly through Kupffer cells, and the space of Disse. Once a sporozoite enters into the liver parenchyma it traverses several hepatocytes, where it receives other signals to achieve full activation for productive invasion. The sporozoite infects a final hepatocyte with formation of a parasitophorous vacuole.

invasion of hepatocytes. Such a role is confirmed by the observation that the migratory activity of *CDPK-6* knockout *P. berghei* parasites was significantly enhanced whereas their ability to productively invade Hepa 1-6 cells was significantly decreased relative to wild-type parasites. Furthermore, *CDPK-6* mutant parasites display a marked decrease in their ability to cleave CSP, further supporting a role for CDPK-6 in invasion.

Whether or not there is an absolute requirement for sporozoite migration through hepatocytes prior to productive invasion is, at present, not entirely clear. It has been shown that migration leads to the release of hepatocyte growth factor (HGF), which acts as a mediator of signals that make the host cell more permissive to development of infection (Carrolo et al., 2003). Another study showed that the treatment of sporozoites with ionomycin, a Ca^{2+} ionophore that stimulates the secretion of apical organelles, leads to enhanced infectivity and inhibition of migration. Also, incubation of sporozoites with lysates of hepatocytes had a similar effect, suggesting that host cells carry molecules that can activate sporozoites for infection (Mota et al., 2002). More recently, it has been shown that exposure of sporozoites to the intracellular K^+ concentration enhances sporozoite infectivity, while decreasing cell migration activity (Kumar et al., 2007). However, Ishino et al., (2004) demonstrated that parasites deficient in a protein named "sporozoite protein essential for cell traversal" (SPECT) do not migrate through cells and yet retain their ability to accomplish infection in vitro, thus questioning the requirement of migration for completion of infection. By showing that a high degree of HSPG sulfation promotes the switch to an invasive phenotype, Coppi et al. (2007) have identified what seems to be the primary trigger to initiate sporozoite activation for productive invasion. However, as these authors point out, despite the fact that hepatocytes contain highly sulfated HSPGs, sporozoites do migrate through several of them before productively invading a final one, suggesting that activation is likely a continuous process that involves the contributions of multiple signaling factors.

The observations of Coppi et al. (2007), however, do not fully resolve the apparent discrepancy between the various observations of increased infectivity as a result of cell migration and the *spect* mutant phenotype. One possible explanation for the *spect* mutants' ability to invade without prior migration through cells is that, rather than being unable to traverse cells, they might be, "by default," activated for invasion. Whichever the case, it seems clear that, under physiological conditions, wild-type sporozoites do migrate through several hepatocytes before productively invading one. Whether this reflects the need for additional sporozoite activation signals or a delay in the signaling events induced by highly sulfated HSPGs remains to be fully elucidated.

The liver stage of *Plasmodium* infection is clearly the least understood of the two stages that comprise the parasite's life cycle within its vertebrate host. This is somehow paradoxical if we consider its potential as a target for prophylactic antimalarial intervention. One of the reasons for the current lack of knowledge is the complexity of

the molecular events that take place in the infected liver. Furthermore, it is becoming increasingly apparent that the vertebrate host does not play a passive role in the infection process and that, on the contrary, it actively influences the fate of infection. In this context, the paper by Coppi et al. (2007) represents a crucial piece of this immense jigsaw.

REFERENCES

Carrolo, M., Giordano, S., Cabrita-Santos, L., Corso, S., Vigario, A.M., Silva, S., Leiriao, P., Carapau, D., Armas-Portela, R., Comoglio, P.M., et al. (2003). Nat. Med. *9*, 1363–1369.

Coppi, A., Pinzon-Ortiz, C., Hutter, C., and Sinnis, P. (2005). J. Exp. Med. *201*, 27–33.

Coppi, A., Tewari, R., Bishop, J.R., Bennett, B.L., Lawrence, R., Esko, J.D., Billker, O., and Sinnis, P. (2007). Cell Host & Microbe *2*, this issue, 316–327.

Ishino, T., Yano, K., Chinzei, Y., and Yuda, M. (2004). PLoS Biol. *2*, e4. 10.1371/journal.pbio.0020004.

Kumar, K.A., Garcia, C.R., Chandran, V.R., Van Rooijen, N., Zhou, Y., Winzeler, E., and Nussenzweig, V. (2007). Mol. Biochem. Parasitol. *156*, 32–40.

Mota, M.M., Pradel, G., Vanderberg, J.P., Hafalla, J.C., Frevert, U., Nussenzweig, R.S., Nussenzweig, V., and Rodriguez, A. (2001). Science *291*, 141–144.

Mota, M.M., Hafalla, J.C., and Rodriguez, A. (2002). Nat. Med. *8*, 1318–1322.

Pinzon-Ortiz, C., Friedman, J., Esko, J., and Sinnis, P. (2001). J. Biol. Chem. *276*, 26784–26791.

Prudencio, M., Rodriguez, A., and Mota, M.M. (2006). Nat. Rev. Microbiol. *4*, 849–856.

Sinnis, P., and Coppi, A. (2007). Parasitol. Int. *56*, 171–178.

Clathrin: An Amazing Multifunctional Dreamcoat?

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Internalization of cargo by clathrin-mediated endocytosis has been studied extensively. In this issue of *Cell Host & Microbe*, Cossart and colleagues report that a variety of pathogens induce the recruitment of clathrin and other endocytic proteins to sites of pathogen interaction with the cell surface. This recruitment is followed by actin rearrangements in the host cell necessary for the uptake or stable attachment of the pathogen at the cell surface.

The plasma membrane serves as a barrier that separates the cytoplasm from the extracellular milieu. Some molecules, like ions, can enter the cell via channels in the lipid bilayer, while other molecules, like growth factors, bind to cell surface receptors and are taken up by endocytosis. There are several types of endocytosis, with clathrin-mediated endocytosis being the most thoroughly studied. Classical clathrin-mediated endocytosis involves the assembly of a clathrin lattice, cargo recruitment via adaptors, formation and release of the vesicle into the cytoplasm, and targeting of the rapidly uncoated vesicle to a specific site within the cell. Besides the key player clathrin, a large number of accessory proteins have been identified that are important for endocytosis, including the adaptor protein AP-2 and, in most cells, the large GTPase dynamin. In addition, actin and actin-regulating proteins, like the Arp2/3 complex and N-WASP, localize to endocytic sites (Figure 1A) (Engqvist-Goldstein and Drubin, 2003). Clathrin-mediated endocytosis usually involves formation of relatively small vesicles (about 100 nm).

Recently, clathrin has been implicated in several nonclassical events at the plasma membrane. Falk and colleagues suggested a role for clathrin in the internalization of large double-membrane vesicles at the lateral membranes of neighboring cells that are coupled by GAP junctions (Figure 1B) (Piehl et al., 2007). Reduction of clathrin levels by RNAi resulted in reduced internalization of GAP junctional clusters. The authors also showed that this process is actin dependent. Additionally, several reports demonstrated that large particles (>1 μm), like latex beads and even certain bacteria and viruses, can utilize the clathrin-associated endocytic machinery and the actin cytoskeleton to enter cells (Aggeler and Werb, 1982; Van Nhieu et al., 1996). Each of these events involves formation of structures substantially larger than classical clathrin-coated vesicles, suggesting previously unappreciated diversity in clathrin's roles and in its organization while mediating cellular processes.

The so-called "zippering" bacteria, such as *Listeria monocytogenes* and *Yersinia pseudotuberculosis*, express proteins on their surfaces that directly interact with receptors on the surfaces of host cells, leading to the clathrin- and actin-dependent endocytosis of the bacteria. InlA and InlB of *Listeria* are among the most extensively studied bacterial surface proteins. InlA binds to E-cadherin, a cell adhesion molecule involved in the formation of intercellular junctions and epithelial cell polarization (Mengaud et al., 1996).