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PARASITE BIOLOGY

A perfectly timed escape

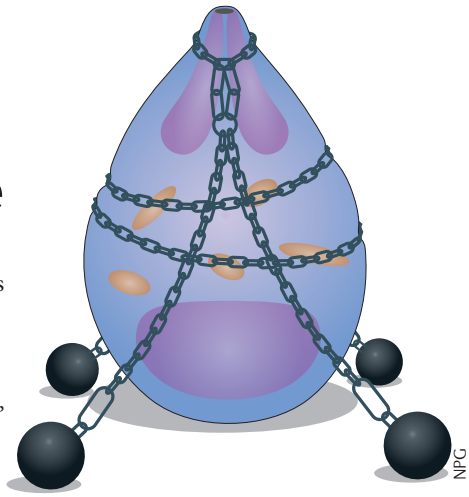
> “processing of … MSP1 by … SUB1 enables MSP1 to interact with the host cell cytoskeleton, enabling egress to occur”

Egress is a poorly understood stage of the *Plasmodium falciparum* life cycle, in which progeny merozoites are released from infected erythrocytes following rupture of the host cell membrane. Now, a new study provides insights into *Plasmodium* egress by showing that processing of merozoite surface protein 1 (MSP1) by the parasite subtilisin-like serine protease SUB1 enables MSP1 to interact with the host cell cytoskeleton, enabling egress to occur.

MSP1 is the most abundant protein on the merozoite surface and is cleaved by SUB1 prior to merozoite egress, but the exact role of MSP1 is unknown. To clarify this, Das *et al.* began by assessing the SUB1-mediated cleavage of recombinant MSP1 (rMSP1) proteins that contained mutations in known and putative processing sites, and determined that the simultaneous mutation of three sites in one protein region was necessary to fully ablate cleavage *in vitro*. To assess the importance of MSP1 processing *in vivo*, the authors used integration constructs to mutate the three cleavage sites in the endogenous *P. falciparum msp1* locus by homologous recombination. Notably, constructs with mutations in one or two of the cleavage sites in MSP1 integrated readily and gave rise to parasites with normal growth, whereas constructs with mutations in all three target sites failed to integrate, suggesting that cleavage of at least one processing site in MSP1 is necessary for parasite viability.

To assess the consequences of SUB1 processing of MSP1, the authors carried out structural studies, which revealed alterations in the secondary structure of rMSP1 after processing by SUB1. Furthermore, cleaved rMSP1 bound more strongly to heparin, permeabilized erythrocytes, inside-out erythrocyte ghost vesicles and erythrocyte cytoskeletons than intact rMSP1. This suggests that SUB1-mediated processing enhances the binding of rMSP1 to an intracellular component of erythrocytes. Further experiments to identify this target revealed that rMSP1 binds to the cytoskeletal protein spectrin, but only after processing by SUB1.

To explore the role of MSP1–spectrin interactions in egress, the researchers chemically inhibited SUB1 to stall parasites at the final stages of schizont development, then measured the kinetics of MSP1 processing and egress following removal of the inhibitor. Parasites that contained a single, weakly targeted SUB1 cleavage site exhibited delayed MSP1 processing, compared with parasites that retained all three cleavage sites. Importantly, this effect was mirrored by a delay in the onset of egress, demonstrating that the kinetics of egress are determined by the rate of MSP1 processing.



Finally, to test the importance of the surface localization of MSP1, the authors generated parasites that inducibly expressed a truncated version of MSP1 that lacked the domain required for tethering to the merozoite surface. Observation of these parasites revealed severe egress defects with incomplete cell membrane rupture and trapping of merozoites in partially ruptured cells, demonstrating that processed MSP1 must be bound to the merozoite surface for egress to occur.

Collectively, these important findings cast new light on *Plasmodium* egress, suggesting a role for mechanical forces exerted from the inside of the infected erythrocyte, aided by interactions between processed MSP1 on the merozoite surface and the spectrin lattice of the host cell cytoskeleton.

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ORIGINAL RESEARCH PAPER Das, S *et al.* Processing of *Plasmodium falciparum* merozoite surface protein MSP1 activates a spectrin-binding function enabling parasite egress from RBCs. *Cell Host Microbe* **18**, 433–444 (2015)