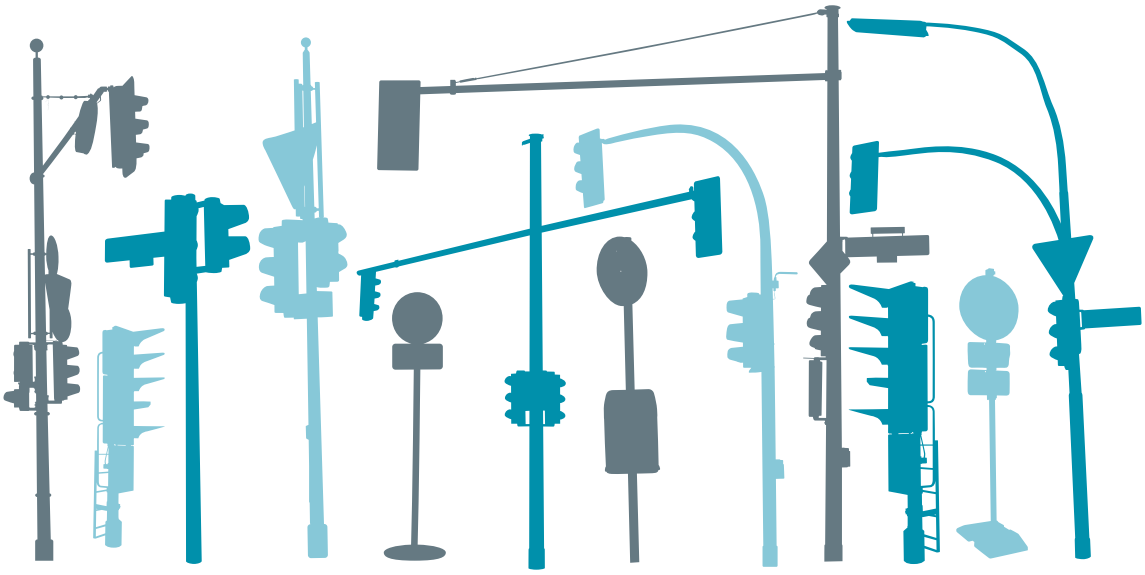

PARASITOLOGY

Multiple signals for apical secretion

Invasion of host erythrocytes by *Plasmodium falciparum* merozoites requires attachment to the erythrocyte surface and the coordinated release of the contents of the apical organelles (micronemes and rhoptries) into the host cell. Publishing in *PLoS Pathogens*, Singh *et al.* now report that distinct and sequential extracellular signals trigger the ordered release of apical organelles during erythrocyte invasion.

Calcium signalling had previously been implicated in regulating motility in *P. falciparum* merozoites, and it has also been linked with the secretion of microneme proteins in *Toxoplasma gondii*, another apicomplexan parasite. Singh *et al.* used time-lapse video microscopy and the calcium-sensitive fluorescence indicator fluo-4 AM to monitor the calcium levels in merozoites during erythrocyte invasion. The high levels of free calcium that were observed in the cytosol decreased following interaction of the merozoites with host erythrocytes. Treatment of merozoites with a calcium ionophore resulted in increased levels of cytosolic calcium together with increased delivery of the microneme proteins erythrocyte-binding antigen 175 (EBA175) and apical membrane antigen 1 (AMA1) to the cell surface. Addition of the membrane-permeable calcium chelator BAPTA-AM blocked the accumulation of cytosolic calcium as well as the surface expression of EBA175, indicating that the level of calcium in the merozoite cytosol is crucial for the secretion of microneme proteins onto the merozoite cell surface.

To investigate the external cues that trigger changes in calcium levels, the authors transferred the merozoites to a buffer that mimicked the physiological conditions found in blood plasma. Using this approach, they observed that increased cytosolic calcium levels and surface expression of EBA175 occurred in response to the low potassium levels in blood plasma. Despite triggering the secretion of microneme proteins, the low-potassium buffer did not lead to the surface expression of rhoptry proteins. The authors postulated that secretion of rhoptry proteins might instead rely on the interaction of the surface-exposed microneme proteins and their cognate receptors on the erythrocyte. Consistent with this idea, interaction of EBA175 with the receptor glycophorin A (GYPA) increased surface expression of the rhoptry protein CLAG3.1 and decreased the levels of cytosolic calcium, suggesting that the interaction between EBA175 and GYPA might be a signal for the restoration of basal intracellular calcium levels and for the secretion of rhoptry proteins to the merozoite cell surface.

Thus, in response to the low potassium levels found in blood plasma, a complex calcium signalling pathway regulates the sequential secretion of proteins from the apical organelles in *P. falciparum*, allowing binding and entry into host erythrocytes.

Andrew Jermy

> “distinct and sequential extracellular signals trigger the ordered release of apical organelles during erythrocyte invasion”

ORIGINAL RESEARCH PAPER Singh, S. *et al.* Distinct external signals trigger sequential release of apical organelles during erythrocyte invasion by malaria parasites. *PLoS Pathog.* **6**, e1000746 (2010)