Arresting malaria parasite egress from infected red blood cells

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The escape of mature malaria parasites from the confines of their host red blood cells is an essential yet poorly understood process. Recent studies now highlight a key role for parasite proteases that trigger the degradation of parasite and host membranes, leading to the egress of infectious parasite forms.

The cyclical waves of fever and chills associated with malaria result from the synchronous rupture of infected red blood cells (RBCs) that release swarms of parasites into the bloodstream for subsequent reinvasion and proliferation. Despite the clinical importance of this phase of the malaria parasite life cycle, relatively little is known about the mechanisms used by the parasite to promote RBC rupture and ensuing egress following the completion of its intraerythrocytic development. Two recent papers, by Arastu-Kapur *et al.*[1] in this issue of *Nature Chemical Biology* and by Yeoh *et al.*[2] in *Cell*, now define key molecular determinants of parasite egress. Using forward and reverse chemical genetic approaches, respectively, these studies arrive at the same conclusion: that a secreted subtilisin-like protease, PfSUB1, plays a central role in what is likely to be a highly regulated proteolytic cascade, leading to the loss of host cell integrity that precedes parasite escape.

The protozoan *Plasmodium* parasites that cause malaria have a complex lifecycle that alternates between human- and mosquito-borne stages. An infective mosquito bite inoculates the human host with a sporozoite form of the parasite that enjoys a brief but productive residence within hepatocytes, followed by release of invasive merozoite forms that target RBCs[3]. Within the RBC, the developing parasite is housed within a membrane compartment called the parasitophorous vacuole that is established during the invasion process (**Fig. 1**). Over the course of 48 h the parasite multiplies to yield ~8–24 daughter merozoites that must destroy their host cell in order to gain access to fresh RBCs and initiate the next round of invasion. A general role for proteases in promoting the release of these daughter cells from the membrane-bound confines of the parasitophorous vacuole and host cell has been known for some time, but the specific enzymes involved have until now remained elusive[4,5].

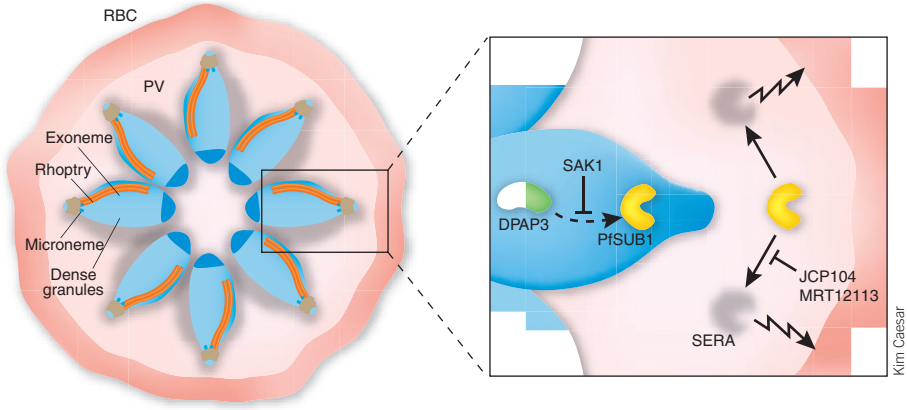

Figure 1 Proteases mediate egress of malaria parasites from the host red blood cell. Parasite development within the RBC occurs within a parasitophorous vacuole (PV). After several rounds of division, ~8–24 mature merozoites breech the PV and RBC membranes. Central to this process of parasite release is the regulated secretion of PfSUB1 into the PV from the exoneme, a novel organelle that is distinct from the apical organelles (rhoptries, micronemes and dense granules) that function during invasion. Secreted PfSUB1 processes the abundant papain-like SERAs, which in turn likely act on membrane components of the PV, and perhaps the RBC, to initiate host cell rupture. Perturbation of PfSUB1 activity, either by direct inhibition with JCP104 (ref. 1) or MRT12113 (ref. 2) (solid arrows), or indirectly by preventing maturation via the DPAP3 inhibitor SAK1 (ref. 1) (dashed arrow), blocks parasite escape from the infected RBC.

PfSUB1 is one of three subtilisin-like proteases expressed by *P. falciparum*, and Yeoh *et al.*[2] have localized this enzyme to a previously undiscovered organelle—the exoneme—from where it is secreted into the parasitophorous vacuole space shortly before host cell rupture. The essential nature of proteolytically active PfSUB1 was demonstrated using transfection-based genetic approaches. To investigate its function, the authors used a high-throughput screen of 170,000 compounds with recombinant PfSUB1 to identify a specific inhibitor, MRT12113. Remarkably, when added to cultured parasites, this inhibitor abrogated host cell rupture, and the few merozoites that were released proved less competent for reinvasion (**Fig. 1**). These data suggest a dual role for PfSUB1 in mediating egress and reinvasion.

These findings by Yeoh *et al.*[2] dovetail nicely with the work by Arastu-Kapur *et al.*[1], who screened a focused library of ~1,200 covalent protease inhibitors for compounds that block parasite release. Starting with a FACS-based assay that detected the increased DNA content of unruptured RBCs full of waiting merozoites, this work identified one serine protease inhibitor (the biotin-tagged chloroisocoumarin JCP104) and two cysteine protease inhibitors (both dipeptide vinyl sulfones) that specifically prevent parasite egress. Aided by the irreversible binding of these inhibitors, Arastu-Kapur *et al.*[1] identified PfSUB1 as the target of JCP104. The authors then used a biotin-labeled FY01 probe, closely related to their cysteine pro-

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tease inhibitors, to identify an unsuspected target—*P. falciparum* dipeptidyl peptidase 3 (DPAP3), a parasite ortholog of human cathepsin C. Competition experiments confirmed that their cysteine protease inhibitors target DPAP3 and the related protease DPAP1. Chemical refinement produced a SAK1 inhibitor that bound to DPAP3 (although some cross-reactivity with other proteases was also noted), and that produced a dose-dependent accumulation of mature, unruptured parasite stages. Further studies suggested that DPAP3 might contribute to parasite egress by regulating the maturation of PfSUB1 (**Fig. 1**). The effects of SAK1 on the maturation of a distinct protein, AMA-1, raises the possibility that DPAP3 may function more generally in the modification of secreted proteins. In the course of pursuing this intriguing question further, it will also be important to eliminate the possibility that SAK1 acts on enzymes other than DPAP3 within the secretory pathway.

In probing how PfSUB1 triggers host cell rupture and parasite release, both teams used their inhibitors to pinpoint another family of putative proteases, the serine repeat antigens (SERAs), as targets of PfSUB1 (**Fig. 1**). The SERAs comprise a family of nine secreted, proteolytically processed proteins with papain-like central domains[6]. Several of these, including the highly abundant SERA5, are delivered to the parasitophorous vacuole in precursor form late in parasite development, where they can be acted on by PfSUB1. Arastu-Kapur *et al.*[1] showed that JCP104 and SAK1 both result in an accumulation of SERA5 precursors, whereas Yeoh *et al.*[2] used their specific MRT12113 inhibitor to demonstrate that native purified SERA4, SERA5 and SERA6 are targets of PfSUB1. Both groups propose that it is the release of PfSUB1 into the parasitophorous vacuole to proteolytically activate the SERAs that triggers the pathway leading to egress.

SERAs may be the major effectors of parasitophorous vacuole membrane destruction, or additional members of the demolition crew activated by PfSUB1 may remain to be identified. Furthermore, it is important to note that most of the SERAs have not been definitively shown to have proteolytic acti-vity, and we are yet to understand the precise mechanisms by which they influence parasite egress. Do they act as a sledgehammer to the foundations of the cell by indiscriminately degrading the membrane components of the parasitophorous vacuole, or do they perform a more surgical dismantling that involves the hydrolysis of specific structural proteins or phospholipids combined with the activation of lipases and pore-forming proteins? New chemical probes specific for the SERA family may help address these questions. Furthermore, such inhibitors may also shed light on parasite egress from host cells at other life stages where proteases also contribute to parasite release. Indeed, gene disruption experiments have demonstrated that expression of the SERA8 homolog in the rodent malaria parasite *Plasmodium berghei* is essential for oocyst rupture in the mosquito midgut[7]. SERAs are also expressed in the liver stages, where cysteine proteases have been implicated in the process of parasitophorous vacuole membrane destruction before parasite egress[8,9].

These studies by Arastu-Kapur *et al.*[1] and Yeoh *et al.*[2] elegantly demonstrate the utility of chemical genetics in understanding basic biological processes in *P. falciparum*, which is notoriously recalcitrant to classical genetic approaches. Finally, the ability of small molecules to perturb specific functions within the parasite may not only provide biological insight but may also create new possibilities for therapeutic intervention.

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It's a mod mod tRNA world

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Postsynthetic modifications are widespread in genetic regulation. Trm9-mediated modification of the anticodon wobble base of specific tRNAs modulates expression of DNA damage response mRNAs in which cognate codons are unusually overrepresented. Thus, modification-dependent tRNA decoding activity is keyed to codon use in a genetic program.

Deciphering the contribution of an individual gene product in a programmed genetic response can provide unique insight. An example is the *Saccharomyces cerevisiae* tRNA anticodon methyltransferase Trm9, deletion of which increases sensitivity to DNA damage. Its activity is related to an emerging field of genetics that focuses on the idea that codon usage in particular mRNAs can be linked to the decoding capacity of specific tRNAs, thereby providing potential for new layers of translational control[1,2]. Though it is clear that limits to the amount of protein produced from mRNAs that bear rare codons can be overcome by increasing levels of the cognate tRNAs, it has not been demonstrated that tRNA levels could be regulated to match codon use by specific mRNAs as part of a genetic program. Using a new computational and bioinformatics approach, Begley *et al.*[3] reveal that certain *S. cerevisiae* mRNAs contain large overabundances of specific codons and deficiencies of others, and they are therefore 'keyed' to rely on the two tRNAs modified by Trm9.

The stereochemistry of mRNA decoding supports involvement of modified uridines in either conventional Watson-Crick U-A base pairs, or depending on the modification, U•G, U•U and U•C wobble pairings, which account for ~30% of decoding. For example, there are six codons for arginine, but only four *S. cerevisiae* $tRNA^{Arg}$ anticodons to decode them; some of these four must

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