Spotlight

Elucidating Host Cell Uptake by Malaria Parasites

Brendan Elsworth,[1]
Caroline D. Keroack,[1] and
Manoj T. Duraisingh[1,*]

The malaria parasite must digest host cytoplasm for normal growth, and many studies have revealed the essential role of proteases in hemoglobin digestion. Here, we discuss the results of Jonscher *et al.* (*Cell Host Microbe* 2019;25:166–173) who have, for the first time, identified a molecule, VPS45, involved in the uptake and trafficking of host cytoplasm to the digestive vacuole.

Malaria, caused by infection of mammalian hosts with parasites of the genus *Plasmodium*, remains a significant cause of morbidity and mortality. Despite decades of research, many of the fundamental biological processes in *Plasmodium* remain enigmatic. A dramatic step in parasite growth is the uptake of up to 80% of the host cell's cytoplasm. However, little is known about the molecular mechanisms underlying this critical process.

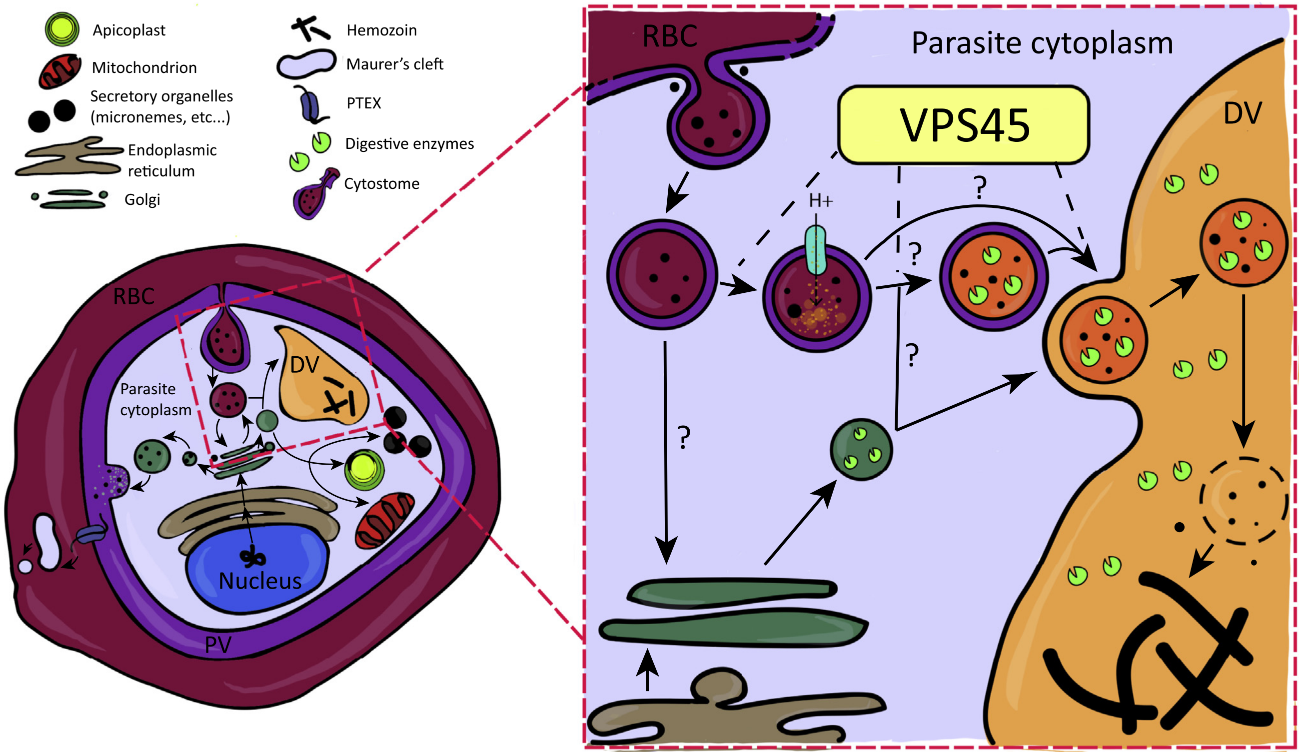
Malaria parasites absorb and degrade the contents of the host cell's cytoplasm, primarily consisting of hemoglobin, to provide nutrients for parasite growth [1]. Uptake of host cytoplasm to provide the space for parasite growth, which eventually grows to fill ~50% of the host cell, and to balance the osmolarity of the cell, may also be necessary [2,3]. Work in the related apicomplexan, *Toxoplasma gondii*, suggests that host cell cytosol uptake (HCCU) may be important for growth and pathogenicity throughout apicomplexan parasites. *T. gondii* undergoes HCCU by a poorly characterized endocytic pathway that transports host proteins to the vacuolar compartment where they are digested by parasite proteases, similar to the process in *Plasmodium* [4].

The mechanism by which *Plasmodium* acquires and transports host cell cytoplasm into the digestive vacuole (DV), where it is degraded by parasite enzymes, has been based primarily on microscopy studies. Several models of HCCU have been described to explain how host cytoplasm is able to cross three membranes – the parasitophorous vacuole membrane (PVM), the parasite membrane (PPM), and the DV membrane – to reach the DV. These include a version of micropinocytosis, with a 'big gulp', whereby ring-stage parasites fold around a large portion of host cytoplasm to form the DV, as well as several variations of an endocytosis-like model [5,6]. In the most accepted model, HCCU occurs through an endocytosis-like pathway through parasite cytostomes. These invaginations occur at the PPM/PVM which then form vesicles that mature into the initial DV or fuse with the formed DV in young and mature parasites, respectively (Figure 1) [5,6].

Previous studies hypothesized that homologs of the endolysosomal pathway may be involved in HCCU in apicomplexans; however, to date, all studied proteins instead play a role in the secretory pathways [7]. Jonscher *et al*. have, for the first time, identified a parasite molecule required for HCCU in *Plasmodium falciparum*. VPS45 belongs to the Sec1/Munc18 family, which, in other eukaryotic organisms, interacts with SNARE proteins that have been implied to be part of *P. falciparum* HCCU in inhibitor studies and is required for membrane fusion of vesicles [8,9]. In *P. falciparum*, VPS45 localizes to foci proximal to the nucleus as well as surrounding the DV, suggesting a role in trafficking and HCCU [8]. Building on previous work by the same group, the authors used a 'knock-sideways' approach in which the target protein is rapidly and inducibly mislocalized from its natural localization within the cell to another, in this case, from the cytoplasm to the nucleus, rendering it nonfunctional [10]. When VPS45 was targeted in *P. falciparum*, parasites were unable to proliferate beyond the trophozoite stage, when hemoglobin digestion is known to be essential [8].

Closer examination of VPS45-inactivated parasites revealed the presence of numerous vesicles within the parasite, which, based on density by electron microscopy (EM), appeared to contain host cell cytoplasm. This was confirmed by preloading host cells with fluorescent dextran, which was taken into the vesicles inside the parasite. By selectively permeabilizing the host cell, the authors showed that these structures were true vesicles and not large invaginations of the PPM/PVM still connected to the host cell, as previous models have suggested. Interestingly, the vesicles formed following inactivation of VPS45 were not entirely uniform, with several large vesicles containing smaller ones, and others enclosed by either a single or double membrane. The presence of double-membrane structures is consistent with the model of an initial invagination step at the PPM that also envelopes the surrounding PVM; however, the presence of single-membrane structures is less clearly explained by current models (Figure 1). While it cannot be ruled out, the heterogeneity may result from the limitations of EM; these heterogeneous vesicles may serve different roles within the parasite, or alternatively may represent different levels of maturation between ingestion and fusion with the DV, or a role for VPS45 in multiple trafficking pathways (Figure 1).

Previous studies have suggested that endocytic vesicles, presumably in transit from the PPM to the DV, were acidified, and that they contained proteases known



Trends in Parasitology

Figure 1. Model of the Trafficking Pathways in *Plasmodium falciparum*. Left. Model of vesicular trafficking pathways in a *P. falciparum*-infected red blood cell (RBC). Proteins destined for parasite organelles, including the digestive vacuole (DV), are trafficked either directly from the endoplasmic reticulum (ER) or through the Golgi before reaching their destination. Proteins destined for the parasitophorous vacuole (PV) and RBC are trafficked through the Golgi and secreted into the PV, where they are transported across the PV membrane by a translocon to the RBC cytoplasm, with a subset of proteins being further trafficked through Maurer's clefts before reaching their final destination in the infected RBC. Uptake of host cytoplasm occurs via an endolysosomal-like system, and host cytoplasm is trafficked to the DV for degradation. Retrograde transport and sorting may occur between the endolysosomal-like system and the Golgi. Right. Model of host cell cytosol uptake and trafficking to the parasite DV. Host cytoplasm is absorbed into the parasite via the cytostome to form endocytic vesicles and is trafficked to the DV. Fusion of the endocytic vesicle with the DV releases host hemoglobin that is then digested by parasite enzymes. It remains unclear if endocytic vesicles mature, including acidification and trafficking of digestive enzymes into the vesicle, prior to fusion with the DV. VPS45 is required for fusion of the endocytic vesicle with the DV, but the exact step in which it acts remains to be determined (shown with dotted lines).

to digest hemoglobin and, in some cases, showed early stages of hemozoin formation, the product of hemoglobin digestion [5]. In VPS45-inactivated parasites, hemozoin was not observed in the vesicles, suggesting that digestion does not occur prior to fusion with the DV. It remains to be determined if some maturation of the vesicles, such as acidification and early steps of hemoglobin digestion, have occurred or are indirectly affected by inactivation of VPS45, such as by preventing trafficking of digestive enzymes to these vesicles (Figure 1). In line with a possible block in maturation, PI3P localized to the induced vesicles, reminiscent of early endolysosomes in other organisms, although this process may vary significantly in *Plasmodium* [9].

Significant research has identified parasite proteases that mediate hemoglobin digestion in the DV, and their drugability. However, this is the first study that identifies a molecular component of the trafficking pathway of host cytoplasm to the DV. This opens the door for the identification of the remaining components of the pathway, which have so far remained elusive, and for the exploration of the essential biology of HCCU. Many unanswered questions relating to HCCU remain. Does VPS45 act at the fusion of vesicles to the DV or at an earlier step? What other parasite molecules are required for HCCU? Has there been significant enough divergence between higher eukaryotes to design specific inhibitors of *Plasmodium* HCCU? How do endocytic vesicles mature prior to fusion with the DV? Will HCCU-defective mutants reveal diverse and unexplored biological processes in both *Plasmodium* spp. and other apicomplexan parasites? Using VPS45 as an entry point into the mechanisms of HCCU will hopefully answer these questions and open up many new ones.

Acknowledgments

Brendan Elsworth was supported by an Australian National Health and Medical Research Council C.J. Martin fellowship (No. 1148392). Caroline D. Keroack was supported by a National Institute of Health, USA, training grant (No. 5T32AI49928-13).

[1]Harvard T.H. Chan School of Public Health, Boston, MA, USA

*Correspondence:
mduraisi@hsph.harvard.edu (M.T. Duraisingh).

https://doi.org/10.1016/j.pt.2019.03.005

References

1. Liu, J. *et al.* (2006) *Plasmodium falciparum* ensures its amino acid supply with multiple acquisition pathways and redundant proteolytic enzyme systems. *Proc. Natl. Acad. Sci. U. S. A.* 103, 8840–8845
2. Hanssen, E. *et al.* (2012) Soft X-ray microscopy analysis of cell volume and hemoglobin content in erythrocytes infected with asexual and sexual stages of *Plasmodium falciparum*. *J. Struct. Biol.* 177, 224–232
3. Lew, V.L. *et al.* (2003) Excess hemoglobin digestion and the osmotic stability of *Plasmodium falciparum*-infected red blood cells. *Blood* 101, 4189–4194
4. Dou, Z. *et al.* (2014) *Toxoplasma gondii* ingests and digests host cytosolic proteins. *mBio* 5, e01188-14
5. Bakar, N.A. *et al.* (2010) Digestive-vacuole genesis and endocytic processes in the early intraerythrocytic stages of *Plasmodium falciparum*. *J. Cell Sci.* 123, 441–450
6. Milani, K.J. *et al.* (2015) Defining the morphology and mechanism of the hemoglobin transport pathway in *Plasmodium falciparum*-infected erythrocytes. *Eukaryot. Cell* 14, 415–426
7. Jimenez-Ruiz, E. *et al.* (2016) Vacuolar protein sorting mechanisms in apicomplexan parasites. *Mol. Biochem. Parasitol.* 209, 18–25
8. Jonscher, E. *et al.* (2019) PfVPS45 is required for host cell cytosol uptake by malaria blood stage parasites. *Cell Host Microbe* 25, 166–173
9. Wallroth, A. and Haucke, V. (2018) Phosphoinositide conversion in endocytosis and the endolysosomal system. *J. Biol. Chem.* 293, 1526–1535
10. Birnbaum, J. *et al.* (2017) A genetic system to study *Plasmodium falciparum* protein function. *Nat. Methods* 14, 450

Spotlight

Mosquitoes on a Diet Reduce Those Pesky Bites

Sharon Hill[1,2] and
Rickard Ignell[1,2,*]

Targeting the internal regulation of mosquito's human-seeking capacity provides a novel means for vector control. Mosquitoes bite us to obtain blood in order to develop their eggs. Professor Vosshall and colleagues (*Cell* 2019;176:687–701) have exploited the modulatory pathway to control this hunger for blood by designing drugs based on human diet-suppression pharmaceuticals.

The capacity of female mosquitoes to transmit disease is intimately linked to their ability to locate humans. Following a blood meal, their ability to locate humans is potently inhibited during a short- and a long-term phase, lasting up to 1 and 4 days, respectively [1]. While the first phase appears to be regulated by stretch receptor feedback from the replete abdomen, previous studies have implicated neuropeptides in initiating and sustaining the long-term inhibition of blood feeding [1]. Artificial manipulation of this inhibition of blood feeding thus provides a new avenue for the development of vector-control tools. While this may seem an obvious strategy, bioavailability, pharmacokinetics (the process of absorption, metabolism, and excretion of drugs), and short half-lives of neuropeptides, along with the perceived redundancy of neuropeptides in their bioactivities, have made this endeavour challenging [2]. Indeed, the recent study by Vosshall and colleagues [3] is the first in which this approach has been successful in a disease vector.

The neuropeptide Y (NPY) pathway in diverse animals, such as humans and fruit flies, is involved in suppressing hunger and food seeking [4]. In the yellow fever mosquito *Aedes aegypti*, injection of NPY receptor activators, head peptide-I (HP-I) and short neuropeptide F (sNPF), transiently inhibits host-seeking behaviour [5–7]. Vosshall *et al*. [3] screened ten human NPY receptor drugs, which are commonly used to control obesity, and found two agonists (small molecules that bind and activate a receptor) of host-seeking behaviour in *Ae. aegypti*. Previous attempts by Vosshall and colleagues to disrupt NPY signalling by targeting one of the eight NPY receptors (NPYLR1) through loss-of-function mutagenesis had no effect on host seeking and blood feeding [5]. Their conclusion, at that time, was that the NPY regulation of host seeking was likely a result of additional receptors, neuropeptides, and their interactions.

To address this potential for functional redundancy and multireceptor–ligand interactions, Vosshall *et al*. [3] screened all 49 neuropeptide receptors identified in the genome of *Ae. aegypti* with all 61 neuropeptide ligands previously detected in adult females [8]. In addition, the receptors were screened with the behaviourally active human NPY receptor drugs, which identified NPYLR7 as the primary target for modulating host seeking after a blood meal [3]. To identify agonists of this receptor that avoid 'off target' effects, particularly on human NPY receptors, Vosshall *et al*. [3] screened 265 211 small molecules in a high-throughput cell assay and identified six candidates. Using a loss-of-function mutant of NPYLR7, the authors subsequently confirmed that this NPY receptor is responsible for the observed second phase of suppression of host seeking after a blood meal. The mutants lacked this second phase and were resistant to the identified agonists. Additionally, nonmutant females, when fed with the identified agonists in nonprotein saline meals, behaved as if they had taken a blood meal, displaying both phases of reduced attraction to humans and no interest in imbibing blood.

The continuing development of insecticide resistance has resulted in the need to discover and develop novel control tools to be used for integrated pest management. Insect neuropeptide receptors are prime targets for this purpose as they