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How *Toxoplasma* and malaria parasites defy first, then exploit host autophagic and endocytic pathways for growth

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Infections caused by the apicomplexan parasites *Plasmodium* and *Toxoplasma* are wide-spread, life-threatening and therapeutically challenging. These pathogens are obligate intracellular microorganisms that invade mammalian cells by forming a self-made niche, the parasitophorous vacuole that is impervious to host lysosomal fusion. Shortly after invasion, a noncanonical xenophagic pathway resembling LC3-associated phagocytosis is activated by the host cell to control infections. However, *Plasmodium* and *Toxoplasma* have elaborated strategies to avoid clearance by the sentinel activities of the host autophagic system. After this initial confrontation, replicating *Plasmodium* and *Toxoplasma* adeptly usurp, for their own benefit, host autophagic and endocytic structures by attracting these organelles to their vacuole, likely to access their nutrient-rich content. The pleomorphic function of the autophagy system, from microbial defense to nutrient supply, is reflected by its ambivalent role during the intracellular development of these apicomplexan parasites.

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Introduction

Plasmodium parasites cause malaria which is responsible for considerable morbidity and, according to WHO (www.who.int/gho/malaria), ~429 000 deaths in 2016. Humans become infected by a female Anopheles mosquito carrying *Plasmodium* parasites (sporozoite form) whose bites deposit sporozoites in the skin from where they invade blood vessels. The parasites passively migrate to the liver, infect hepatocytes wherein they undergo asexual reproduction (schizont liver form), then escape from hepatocytes and invade red blood cells (merozoite form) where an additional round of replication takes place. Among species that are recognized to infect humans in nature, *Plasmodium falciparum* is found worldwide in tropical and subtropical areas, predominating in Africa whereas *Plasmodium vivax* is found mostly in Asia, Latin America, and in some parts of Africa. *P. falciparum* is the deadliest malaria species, causing cerebral malaria associated with anemia, coma and death. Unlike *P. falciparum* that massively populates the blood, *P. vivax* is less virulent as it can go dormant (hypnozoite form) in the liver for days to years, causing no symptoms; however, chronic and recurrent episodes of *P. vivax* infections can lead to organ failures and cerebral malaria [1]. *Toxoplasma gondii*, the infectious agent of toxoplasmosis, is one of the most ubiquitous human pathogens found worldwide, potentially causing fatale encephalitis in immunocompromised individuals [2]. Humans contract *T. gondii* by oral ingestion of parasite cyst forms that transform into a rapidly multiplying stage (tachyzoite form) in enterocytes before dissemination throughout the body and encystation (bradyzoite form) in the brain and muscle cells.

Plasmodium sp. and *T. gondii* are obligate intracellular parasites, and host cell entry is paramount to their survival. *Plasmodium* parasites exhibit a high level of host cell specificity as liver forms and blood forms invade solely hepatocytes and erythrocytes, respectively. Conversely, *Toxoplasma* is a generalist, able to invade any nucleated cells of warm-blooded animals; during natural infections in humans, the parasite can enter and survive in hematopoietic cells (e.g. monocytes, dendritic cells) and in a variety of nonhematopoietic cells (e.g. enterocytes, endothelial cells, brain cells, cardiocytes, skeletal cells, retinal pigment epithelial cells) [3–5]. After a successful invasion, regardless of host cell type, these apicomplexan parasites, ensconced in their parasitophorous vacuole (PV) face the risk of being besieged and eliminated by host innate defense responses. Surviving parasites derive benefit from the nonselective autophagic pathway in the host cell to support their growth [6,7].

The first part of this review will center on the recognition and capture of the PV by a unique host selective xenophagic process, and how *T. gondii* and *Plasmodium* have evolved to subvert this harmful defense mechanism in the inimical host cell. In the second part, I will address the strategies developed by *Toxoplasma* and *Plasmodium* to

exploit the autophagic and heterophagic functions of their host cells. For this review, we will focus on *T. gondii* and *Plasmodium* liver stage, with special reference to *P. vivax* and the rodent model malaria parasite *Plasmodium berghei*.

The tricks of successful host cell invasion by *Toxoplasma* and *Plasmodium*

Apicomplexan parasites use a unique form of actin-myosin based motility to rapidly invade their host cell, avoiding the risk of phagocytic engulfment [8]. For penetration, *T. gondii* tachyzoites rely on a firm anchorage to their host cell mediated by proteins, secreted from microneme and rhoptry organelles, which assemble to form a structure called the moving junction. The moving junction forms a tight connection between the *Toxoplasma* and host cell plasma membranes, and moves from the apical to posterior end of the parasite thereby invaginating the host plasma membrane as *Toxoplasma* progresses into the cell [9]. Remarkably, the moving junction acts a molecular sieve, forming a physical barrier that excludes all transmembrane proteins and most GPI-linked proteins from the nascent PV membrane [10]. This results in a PV lacking receptors susceptible to intersect with host intracellular trafficking machinery and, thus, incompetent to fusion with endo-lysosomes. Following host cell penetration, *Toxoplasma* designs the composition of its PV membrane by incorporating its own proteins and lipids, thus making the PV a unique, specialized 'organelle' in infected cells. Some of the parasite-derived proteins at the PV surface act as recruiters of host ER tubules and mitochondria, forming molecular interactions between these organelles and the PV membrane [11–13]. This dense covering of mammalian organelles further contributes to disguise the PV into an 'incognito' compartment within the host cytoplasm.

Similarly to *Toxoplasma* tachyzoites, *Plasmodium* sporozoites actively invade hepatocytes and form a PV membrane [14]. Liver cell recognition is specifically mediated by both parasite proteins and host cell receptors [15–17]. There is no experimental evidence that *Plasmodium* sporozoites establish a moving junction during entry, which would allow the exclusion of components of the host cell surface from the PV membrane. Nevertheless, the vast majority of the PV escapes fusion with host degradative organelles. The PV membrane contains several parasite-derived proteins [18] and interacts with host ER [19].

Act I: 'Embracing the enemy': how the host cell spots the PV and marks it for elimination

The rapid process of active invasion mediated by *Toxoplasma* and *Plasmodium* accompanied by the formation of a nonfusogenic PV preserves these parasites from immediate phagocytic clearance. However, as early as 2 min post-infection, host LC3 or homologs (GABARAPs) are detected at the surface of *T. gondii* and intrahepatic *Plasmodium* vacuoles, indicating the detection of these intruders by the host cell autophagy machinery [20•,21•,22•,23,24••,25••,26,27••] (Figure 1). In mammalian cells, degradative autophagy plays crucial roles in immune defense against intracellular pathogens ([28]; see review by E.M. Frickel and J. Saeij in this issue). Correspondingly, parasite growth is stunted in LC3-positive vacuoles. The association of LC3 with the PV membrane results from the direct incorporation of lipidated LC3 in this membrane and not from the fusion of LC3-positive autophagosomes with the PV [26]. This process is reminiscent of the noncanonical autophagic pathway named LC3-associated phagocytosis (LAP), in which LC3 localizes to single-membrane phagosomes containing extracellular pathogens or dead cell corpses, and contrasts to canonical autophagy in which LC3 is recruited onto double-membrane autophagosomes [29–31]. In canonical LAP, LC3 marks phagosomes destined for lysosomal fusion, thus creating an autophagolysosome. LAP depends on the specific downstream autophagic proteins, for example, Beclin 1/ATG6, phosphatidylinositol 3-kinase (PI3K), ATG5, and upstream autophagic proteins, for example, ATG1. However, some of these effectors are not operative for LC3 recruitment at the PV of *Plasmodium* in hepatocytes, such as Beclin 1/ATG6 and PI3K for *P. berghei* [26] and ATG1 for *P. vivax* [25]. Thus, an unconventional LAP is operational in hepatocytes to control malaria infection.

Upstream of the LC3 labeling of the *T. gondii* and *Plasmodium* PV, the LC3 conjugation system including the ATG12-ATG5-ATG16L1 complex, is recruited to the PV membrane [20•,21•,26,32,33]. This complex functions as an E3-like ligase, and in concert with the E1-like activating enzyme ATG7 and the E2-like conjugating enzyme ATG3 [31], it conjugates LC3 to a phospholipid for membrane anchorage. In infected mammalian cells lacking *Atg5*, *Atg3* or *Atg16l1*, the PV labeling with LC3 is dramatically decreased. The nature of the factor/s guiding ATG7, ATG3 and the ATG12-ATG5-ATG16L1 complex to the PV is still unknown. It is possible that the ATG12-ATG5-ATG16L1 complex targets the PV membrane by recognizing abnormal membranous structures or a pathogen-associated molecular pattern (PAMP). An alternative hypothesis would involve IFNγ-regulated GTPases (IRG); these proteins include effectors of the GKS subfamily (carrying a canonical GxxxxGKS motif in the P-loop of the GTP-binding site) and of the GMS subfamily (with a non-canonical GxxxxGMS motif) [34,35]. GMS IRG are 'protective' proteins that exert a regulatory function over the 'executor' GKS IRG. In the absence of GMS IRG on a given membrane, GKS IRG can diffuse to this membrane and utilize their dynamin-like domains to strip the membrane upon IFNγ activation ('missing-self' model in [36]). By analogy, the ATG12-ATG5-ATG16L1 complex may bind to the PV membrane in the absence of host GMS IRG.