including kinases and phosphatases, leading to pleiotropic functions. Many protozoan parasites require phosphatidic acid for their growth. To this point, phosphatidic acid is both synthesized and salvaged from mammalian cells by the intravacuolar parasite *Toxoplasma gondii*, and this lipid plays a central role in the parasite's cell cycle and infectivity, controlling invasion and egress from the host cell [6,7].

How *Tubulinosema* accesses host phosphatidic acid or other lipids from the LDs of adipocytes needs further investigation. Like *T. ratisbonensis*, several pathogens that infect vertebrates find a home in adipose tissues, multiply in adipocytes, feeding on or exploiting host LDs as part of anti-immunity strategies [8,9]. Mammalian adipocytes subsist for up to 10 years, providing thus a long-term environment for a pathogen, and they offer a copious source of nutrients, thereby presenting many advantages to pathogens. Adipose tissues are targeted by *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Plasmodium falciparum* and constitute a sequestration site during their chronic phase of infection in humans. Many intracellular pathogens such as *T. cruzi*, *Leishmania amazonensis,* and *T. gondii* internalize host LDs into their parasitophorous vacuole, likely to benefit from their neutral lipid content. *T. gondii* retrieves fatty acids stored in host LDs, and the depletion of LDs from the host cell is detrimental for the parasite's growth [10]. Based on these examples, interference with host LD–pathogen interactions could be harmful for the intruder, leading to its death by starvation.

[1]Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

*Correspondence: icoppen1@jhu.edu (I. Coppens).

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Spotlight

Interrupting *Toxoplasma*'s Regularly Scheduled Program of Egress

Vern B. Carruthers[1],*

Although many cellular components of the Ca^{2+} signaling pathway dictating *Toxoplasma gondii* egress have been identified, whether the parasite secretes protein activators of this pathway remained unknown. Bisio *et al.* (*Nat. Microbiol*. 2019;4:420–428) identify a parasite-secreted diacylglycerol kinase as a key upstream activator of signaling for 'programmed' egress from host cells.

Staring through a microscope at cells infected with *T. gondii*, it is easy to think that the parasite simply outgrows its intracellular niche and spills out into the surrounding medium. By contrast, such a ubiquitously successful parasite probably would not leave anything to chance. Indeed, there have been indications that the parasite plays more of an active role in 'natural' egress than originally assumed. Intriguing new work from Dominique Soldati-Favre's laboratory is making it even clearer that *Toxoplasma* programmatically orchestrates its exit from host cells [1].

T. gondii replicates inside a specialized membrane-bound compartment called the parasitophorous vacuole (PV). Most insight about *Toxoplasma* egress has come from inducing egress with treatments that directly or indirectly elevate parasite cytosolic Ca^{2+}. Increased Ca^{2+} drives microneme secretion and gliding motility, which are both required for efficient exit (Figure 1). Micronemes and a second set of secretory organelles, called dense granules, contain membrane-disrupting proteins that facilitate rupture of the PV during egress. Micronemes also harbor transmembrane adhesive proteins that connect with the Ca^{2+}-responsive actinomyosin gliding motility system (glideosome) to provide traction for gliding motility. In this manner, a combination of membrane-active agents and motive force collaborate for escape.

Work over the past several years has steadily identified signal transduction proteins upstream of Ca^{2+} release, leading to a working model of the pathway (Figure 1) (reviewed recently in [2]).

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Figure 1. Working Model of Programmed Egress. The apical region of an intracellular *Toxoplasma gondii* zoite is illustrated to show components of the cyclic nucleotide and Ca^{2+} signaling pathway that regulates egress. The pathway generates phospholipid species, including PA, to dock micronemes to the apical membrane, and IP3, which triggers Ca^{2+} mobilization from the ER to promote microneme secretion and initiate motility for parasite exit. Membrane-disrupting proteins are indicated as pink donuts. Microneme transmembrane adhesive proteins are illustrated as tulips. Abbreviations: APH, acylated pleckstrin homology domain-containing protein; CDPKs, calcium-dependent protein kinases; CD, cell division control protein 50.1 (CDC50.1); DAG, diacylglycerol; DGK1, diacylglycerol kinase 1; DGK2, diacylglycerol kinase 2; ER, endoplasmic reticulum; GC, guanylate cyclase; IP3, inositol triphosphate; HPM, host plasma membrane; Mn, microneme; PA, phosphatidic acid; PDE2, phosphodiesterase 2; PIP2, phosphatidyl inositol diphosphate; PKA, PKA-C1 protein kinase A catalytic domain; PKG, protein kinase G; PLC, phosphatidylinositol specific phospholipase C; PV, parasitophorous vacuole; PVM, PV membrane; TVN, tubulovesicular network; UGO, unique guanylate cyclase organizer.

New work from the Soldati-Favre team identified three novel components of this signaling cascade, including a second diacylglycerol kinase termed DGK2 [1]. DGK2 is secreted from dense granules into the PV, implying a function exterior to the parasite. Parasites conditionally deficient in DGK2 continue replicating inside host cells well beyond the time of normal egress, eventually rupturing out of an overstuffed PV. The team found that exogenously added PA triggered microneme secretion in a GC- and PKG-dependent manner, suggesting that PA acts upstream of cGMP signaling. That a decrease in PV pH, which has been proposed to occur during egress [6], rescued the DGK2 egress defect implies the existence of at least one bypass route. The study also identified two new partners of GC, CDC50.1 and UGO (unique GC organizer). CDC50 family proteins are known to stably associate with P-type ATPase lipid flippases, which is consistent with GC being comprised of two GC domains and a P-type ATPase domain [1,7]. Conditional knockdown of CDC50.1 destabilized and mislocalized GC, resulting in an egress defect mirroring that of DGK2 deficiency. Unlike CDC50.1, UGO is found only in apicomplexan parasites. Parasites conditionally lacking UGO are also defective in the stability and trafficking of GC. Interestingly, indirect evidence suggests that UGO is necessary for GC activity whereas CDC50.1 is not, implying a more important role for UGO in GC production of cGMP. Together, these studies indicate a role for DGK2 and PA upstream of GC, which depends on CDC50.1 and UGO for its function in promoting egress.

The new findings are notable in at least two ways. First, the discovery of DGK2 adds to previous work identifying other dense granule proteins, GRA22 and GRA41, which are required for correct timing of egress [8,9]. Whereas DGK2 deficiency results in delayed egress, disruption of GRA22 or GRA41 manifests in

Briefly, a recently identified guanylate cyclase (GC) generates cyclic guanosine monophosphate (cGMP), which activates a cGMP responsive kinase (protein kinase G, PKG) that positively regulates several downstream components. These include activation of a phosphatidylinositol phospholipase C (PI-PLC) to generate inositol triphosphate (IP3). IP3 triggers release of Ca^{2+} from intracellular stores to stimulate Ca^{2+}-dependent protein kinases (CDPKs) that regulate microneme secretion and motility. PI-PLC also generates diacylglycerol (DAG), which is

phosphorylated by a diacylglycerol kinase (DGK1) to produce phosphatidic acid (PA). A PA-binding protein (APH) on the surface of micronemes engages PA to facilitate membrane docking for microneme discharge [3]. This egress 'accelerator' pathway has a 'braking' mechanism provided by recently discovered adenylate cyclases and a cAMP-responsive kinase (PKA-C1) [4,5]. PKA-C1 was proposed to regulate at least one cGMP phosphodiesterase (PDE2) to limit cGMP and keep the brakes engaged during intracellular replication.

premature egress. Together, these findings indicate that *Toxoplasma* secretes positive and negative regulators of programmed egress. Programmed egress might have evolved as an intrinsic mechanism to ensure maximum growth by departing before fully exhausting host cell resources. The parasite can also hasten egress in an emergency situation by sensing extrinsic factors such as damage to the infected cell causing fluxes of K^+ and Ca^{2+} or engagement by an effector T cell. Second, identification of the GC-CDC50.1–UGO complex and its positioning to a discrete site on the parasite apical surface suggests a need for spatial organization of signaling proximal to the site of microneme exocytosis. That several other components of the signaling pathway are not confined to the apical region during parasite replication opens the possibility of their dynamic repositioning immediately prior to egress.

As with many important advances, the work opens several new questions. (i) Is DGK2 activity suppressed during parasite replication and activated immediately prior to egress and, if so, what controls the timing of DGK2 activity? (ii) What is the basis for the GC complex being specifically confined to the apical surface? (iii) Does PA act through the GC complex, and if so, is it a direct or indirect activator of GC activity? (iv) How do extrinsic cues (immune assault, ion flux) integrate with or bypass the DGK2-dependent signaling pathway? (v) Since a host G-protein-coupled signaling pathway has been implicated in *Toxoplasma* egress [10], is there crosstalk between host and parasite signaling cascades?

It can be expected that future genetic and proteomic studies will identify additional upstream regulators of programmed egress and provide more insight into how this key event is controlled. Although much is yet to be discovered, if the recent pace continues, the parasite's regularly scheduled program of egress will steadily come into view.

Acknowledgments

[1]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-5620, USA

*Correspondence: vcarruth@umich.edu (V.B. Carruthers).

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