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regulate many physiological and behavioural processes [1,2]. While small-molecule agonists have been identified to target human neuropeptide receptors as a novel direction in the drug industry, the approach taken by Vosshall *et al*. [3] is novel in insects. In *Ae. aegypti*, the available high-quality genome assemblies [9], along with a comprehensive identification of bioactive neuropeptides and their receptors [8,9], established the platform for this study. For applied purposes, however, any neuropeptide receptor agonist to be used in vector control requires easy delivery, maintenance of metabolic and environmental stability, as well as being cost-effective [2], aspects only partially addressed by Vosshall *et al*. [3]. In addition, since neuropeptide pathways are highly conserved among insects, off-target effects need to be addressed to limit unwanted consequences.

Species specificity of the tools using agonists, such as the ones identified by Vosshall *et al*. [3], will have to rely on additional components, for example, odour-based attractants [10]. The benefit of the nonspecificity of the agonists themselves increases the importance of the innovation by the authors, and fits well with the objectives of integrated vector-control tool implementation. The development of odour-based attractants has accelerated over the past decade, with numerous floral and host odorants showing efficacy in attract-and-kill strategies in the field [10]. Combining neuropeptide receptor agonists with, for example, floral attractants, suggests their integration with a modified version of the already-available sugar bait technology. As a whole, we believe that the comprehensive analysis provided by the authors will be a valuable source of information for future studies aimed at developing integrated tools for vector control.

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Spotlight

Robbing Host Phosphatidic Acid to Survive: A Strategy of a Fly Parasite

Isabelle Coppens[1,*]

***Drosophila* flies can be infected by an obligate fungal intracellular parasite, *Tubulinosema ratisbonensis*, resulting in a swollen abdomen and often death. Within the fly, the parasite multiplies in the cytoplasm of adipocytes of the fat body, feeds on host lipid droplets, and has a specific requirement for dietary phosphatidic acid.**

Parasites love fat. Parasites deviously scavenge and consume lipids from their hosts. And sometimes, it costs the infected host its life. A prime example is illustrated by the obligate intracellular parasite *T. ratisbonensis* that infects and proliferates in *Drosophila melanogaster* – our major model system in modern biology, resulting in a shortened lifespan and reduced fertility for the infested fly. If kept at 25°C and left uncured, infested *Drosophila* stocks in laboratories can be devastated within a week. To explore strategies to cure or protect *Drosophila* cultures from *Tubulinosema* infection, a better understanding of the systemic effects of an infection on the fly and the identification of host factors exploited by the parasite allowing its successful proliferation, is crucial. By dissecting *Drosophila* metabolic networks through selective feedings, or the silencing of specific metabolic genes, Franchet and colleagues [1] have identified a major contributor to the rapid growth of *Tubulinosema* in the lipid metabolism of the fly.

T. ratisbonensis belongs to the phylum Microspora that comprises spore-forming, intracellular protist parasites that infect vertebrates and invertebrates, even those that are themselves parasites. Microsporidia produce environmentally resistant spores that are equipped with a sophisticated invasion machinery, consisting of an extrusion apparatus that contains a coiled polar tube, that is, a long, flexible, syringe-like structure [2]. During host cell invasion, the polar tube explosively extrudes from the spore, pierces the targeted host cell plasma membrane, and inoculates the host cell with infective sporoplasm, that is, the central mass of cytoplasm within the spore. Then, intracellular sporoplasm matures into a meront that undergoes several rounds of asexual reproduction, leading to the generation of up to several hundred meronts in a single host cell.

As with any microsporidian, *T. ratisbonensis* has lost many essential genes in

response to its adaptation to the lifestyle of intracellular parasitism [3]. While it possesses a nucleus and intracytoplasmic membranous system, it lacks many typical eukaryotic characteristics. For example, the parasite has ribosomes that resemble those of prokaryotes, a primitive mitochondrion (mitosome), an atypical Golgi apparatus, and no peroxisomes. Its genome lacks many genes involved in fatty acid anabolism (e.g., acetyl-CoA carboxylase, fatty acid synthase) and fatty acid β-oxidation (e.g., acyl-CoA oxidase). Reduced to host dependency and scavenging, *Tubulinosema* relies on its own transporter activities to steal host metabolites during its intracellular life stage.

A hallmark of *Drosophila* infection with *Tubulinosema* is the impressive swelling of the fly's abdomen (Figure 1A,B). Within the fly, *Tubulinosema* preferentially proliferates in cells of the fat body, a large organ distributed throughout the fly body (Figure 1C). The fat body plays a major role in intermediate metabolism in insects and is the central storage depot of nutrients and energy reserves [4]. The predominant cell of the fat body is the adipocyte, characterized by the accumulation of numerous lipid droplets (LDs) containing triacylglycerols (TAGs). By targeting a fat body adipocyte (the storage sanctuary) as a host cell to establish infection, the parasite secures for itself a steady and copious supply of nutrients. The choice of adipocytes as sites of multiplication for *Tubulinosema* in the fly gives us a first clue of the parasite's need from the host: likely a biosynthetic or stored molecule, for example, glucose, free fatty acid, glycogen, and/or TAGs. Indeed, as *T. ratisbonensis* meronts expand in the fat body, the LDs of infected adipocytes progressively dissolve, as a consequence of their consumption by the parasite. This 'cellular vampirism' results in a dramatic collapse of the entire fat body lobule structure followed by the fly's death.

Which nutrients, present in adipocytes, does *Tubulinosema* covet? As a first approach to solving this enigma, the authors gave a super-rich meal (yeast) to the infected flies and observed an acceleration of the parasite's proliferation. This confirms a relationship between *Tubulinosema* virulence and a high nutrient density diet of the fly. As a next approach to decipher which particular biochemical compounds coming from the food are responsible for this phenomenon, the authors selectively added amino acids, sterols, fatty acids, or sugars to the standard sucrose solution on which flies feed. Only supplementation with fatty acids, for example, oleate and palmitate, led to a doubling of parasite load. This information leads to a second clue on the parasite's requirement for growth by pointing to polar lipids as the desired nutrients for meronts.

As a third approach to refine which host lipid metabolic processes are required for parasite proliferation, the authors silenced specific genes involved in fatty acid synthesis, transport or β-oxidation, lipid droplet biogenesis or degradation, and regulation of lipid metabolism. Reduced expression of a host intestinal TAG lipase involved in the digestion of dietary TAG impairs parasite replication, establishing that dietary intake of lipids contributes to the virulence of the infection. Markedly, silencing key enzymes involved in the synthesis of phosphatidic acid from fatty acids to its direct glycerol phosphate precursors negatively impacts the meront's growth. Conversely, preventing the use of phosphatidic acid by the fly through blockade of phosphatidylethanolamine, phosphatidylcholine, or TAG synthesis favors the proliferation of *Tubulinosema*. When phosphatidic acid is directly injected into the fly, the parasite load even quadruples. These assays offer the final clue to the parasite's nutritional need from the host by narrowing down the list of dietary lipids hijacked by the parasite to phosphatidic acid.

Why and how phosphatidic acid magically boosts the proliferation of *T. ratisbonensis* in *Drosophila* needs to be investigated. Phosphatidic acid is a peculiar lipid, being both a key building block of phospholipid synthesis and a major second messenger conveying signaling information [5]. With its cone-shaped geometry, phosphatidic acid locally changes membrane topology and plays a role in membrane trafficking and fusion events. It interacts with numerous proteins,

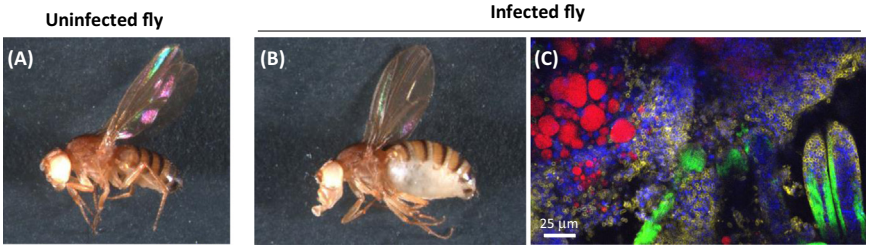

Figure 1. Infection and Colonization of *Drosophila* by *Tubulinosema ratisbonensis*. (A) Illustration of the normal morphology of an uninfected *Drosophila*. By contrast, upon infection of the fly with *T. ratisbonensis* for 9 days as shown in (B), the fitness and body corpulence of the fly is dramatically altered, with a distended, swollen abdomen. *T. ratisbonensis* meronts preferentially target host adipocytes wherein they proliferate and progressively fill the fat body lobule of the fly. (C) Illustration of the area of fat body adipocytes, enriched in lipid droplets (red), that is being invaded. Most of the DAPI signal (blue) originates from the meronts. The yellow staining corresponds to mature spores of the parasites. FITC-actin in shown in green. The figure is courtesy of the D. Ferrandon group from the Université of Strasbourg, France.

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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.

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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]).