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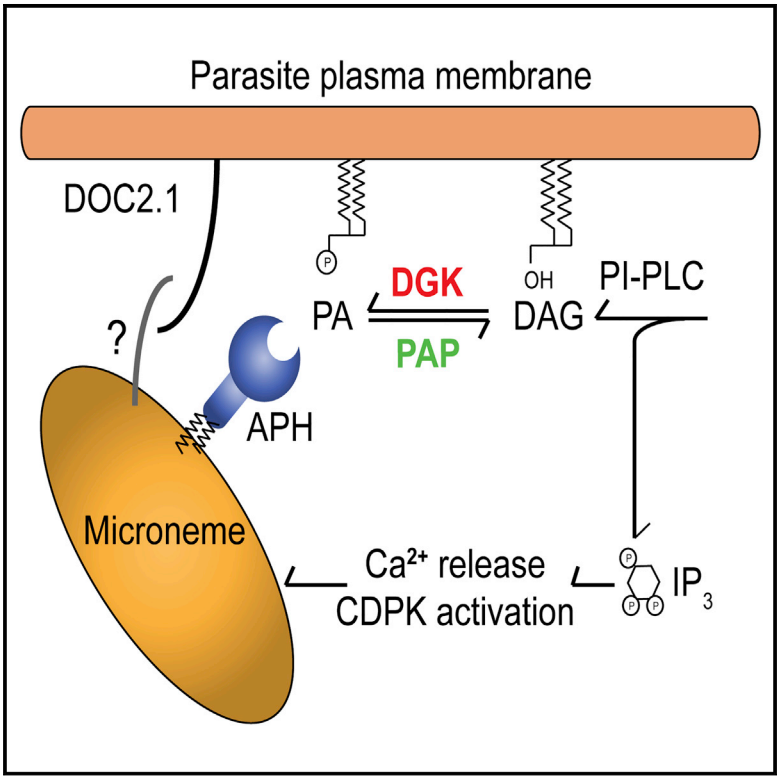
Cell Host & Microbe

Phosphatidic Acid-Mediated Signaling Regulates Microneme Secretion in *Toxoplasma*

Graphical Abstract



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In Brief

Microneme secretion is essential for efficient propagation of Apicomplexan parasites. In this issue of *Cell Host & Microbe*, Bullen et al. (2016) demonstrate that this process is underpinned by phosphatidic acid regulation at the parasite plasma membrane, controlled by the essential enzyme DGK1 and sensed by the microneme protein APH.

Highlights

- Membrane phosphatidic acid (PA) regulation is linked to *T. gondii* microneme secretion
- Diacylglycerol kinase-1 (DGK1) underpins PA generation for microneme secretion
- The microneme surface protein APH detects PA at the parasite plasma membrane
- Both APH and DGK1 are critical for microneme secretion in *T. gondii*

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Phosphatidic Acid-Mediated Signaling Regulates Microneme Secretion in *Toxoplasma*

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SUMMARY

The obligate intracellular lifestyle of apicomplexan parasites necessitates an invasive phase underpinned by timely and spatially controlled secretion of apical organelles termed micronemes. In *Toxoplasma gondii*, extracellular potassium levels and other stimuli trigger a signaling cascade culminating in phosphoinositide-phospholipase C (PLC) activation, which generates the second messengers diacylglycerol (DAG) and IP_3 and ultimately results in microneme secretion. Here we show that a delicate balance between DAG and its downstream product, phosphatidic acid (PA), is essential for controlling microneme release. Governing this balance is the apicomplexan-specific DAG-kinase-1, which interconverts PA and DAG, and whose depletion impairs egress and causes parasite death. Additionally, we identify an acylated pleckstrin-homology (PH) domain-containing protein (APH) on the microneme surface that senses PA during microneme secretion and is necessary for microneme exocytosis. As APH is conserved in Apicomplexa, these findings highlight a potentially widely used mechanism in which key lipid mediators regulate microneme exocytosis.

INTRODUCTION

Active host cell entry is an essential step in the propagation of obligate intracellular parasitism by apicomplexan parasites, members of which include the major etiologic agents of malaria (*Plasmodium spp.*) and toxoplasmosis (*Toxoplasma gondii*). Underpinning this process is the release of apical secretory organelles termed micronemes, secretion of which is a prerequisite for gliding motility, invasion, and egress from infected cells (reviewed in Sharma and Chitnis, 2013). Despite its central role in infectivity, our current understanding of the molecular mechanisms governing microneme secretion is sparse. Microneme exocytosis is known to follow changes in extracellular potassium levels (Singh et al., 2010), implicates cyclic GMP-dependent protein kinase G (PKG) (Brochet et al., 2014) and phosphoinositide regulation (Brochet et al., 2014), and responds to an increase in intracellular calcium concentration (Brochet et al., 2014; Garg et al., 2013; Singh et al., 2010; Singh and Chitnis, 2012; Wiersma et al., 2004). PKG activity promotes formation of the phosphoinositide-phospholipase C (PI-PLC) substrate $PI_{(4,5)}P_2$ (Brochet et al., 2014), implicating PI-PLC as the downstream mediator of PKG activity. Concordantly, *P. falciparum* PI-PLC transcription is upregulated during late blood stages, and the *P. berghei* homolog is refractory to genetic deletion (Raabe et al., 2011). Inhibitor studies suggest that PI-PLC acts on $PI_{(4,5)}P_2$ to generate the second messengers IP_3 and DAG and stimulates Ca^{2+} release from the endoplasmic reticulum (ER) or other internal stores (Singh et al., 2010) through unidentified receptors (reviewed in Budu and Garcia, 2012). Ethanol stimulation of *T. gondii* microneme secretion also triggers an increase in calcium, likely as a result of PI-PLC-derived IP_3 (Carruthers et al., 1999; Lovett et al., 2002). Members of the calcium-dependent protein kinase (CDPK) family are critically involved downstream of this signaling cascade (Garrison et al., 2012; Lourido et al., 2012; McCoy et al., 2012; reviewed in (Holder et al., 2012). Ultimately, a fusion event believed to involve SNARE-like proteins such as DOC2.1 (Farrell et al., 2012; Jean et al., 2014) enacts microneme exocytosis.

With a view to better deciphering microneme exocytosis, we have focused here on diacylglycerol (DAG) and phosphatidic acid (PA), downstream products of PI-PLC signaling at the parasite plasma membrane (PPM). DAG is interconverted to PA via DAG kinases (DGKs) and PA phosphatases (PAPs). In mammalian systems, PA is involved in signal transduction (Chasserot-Golaz et al., 2010), membrane dynamics (Kooijman et al., 2003), and exocytosis (reviewed in Ammar et al., 2013; Chasserot-Golaz et al., 2010), thus providing a precedent for PA to also play a role in microneme exocytosis in the Apicomplexa. We have identified and functionally characterized both the enzyme mediator of PA production and the corresponding PA-sensor underpinning an essential mechanism of microneme exocytosis conserved across the Apicomplexa.

RESULTS AND DISCUSSION

Modulation of PA Levels Plays a Critical Role in *T. gondii* Microneme Secretion

The importance of PA signaling in microneme secretion was implicated by the putative increase in its precursor (DAG) during