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A map of the subcellular distribution of phosphoinositides in the erythrocytic cycle of the malaria parasite *Plasmodium falciparum*

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ABSTRACT

Despite representing a small percentage of the cellular lipids of eukaryotic cells, phosphoinositides (PIPs) are critical in various processes such as intracellular trafficking and signal transduction. Central to their various functions is the differential distribution of PIP species to specific membrane compartments through the actions of kinases, phosphatases and lipases. Despite their importance in the malaria parasite lifecycle, the subcellular distribution of most PIP species in this organism is still unknown. We here localise several species of PIPs throughout the erythrocytic cycle of *Plasmodium falciparum*. We show that PI3P is mostly found at the apicoplast and the membrane of the food vacuole, that PI4P associates with the Golgi apparatus and the plasma membrane and that PI(4,5)P2, in addition to being detected at the plasma membrane, labels some cavity-like spherical structures. Finally, we show that the elusive PI5P localises to the plasma membrane, the nucleus and potentially to the transitional endoplasmic reticulum (ER). Our map of the subcellular distribution of PIP species in *P. falciparum* will be a useful tool to shed light on the dynamics of these lipids in this deadly parasite.

1. Introduction

Despite recent progress in reducing mortality and morbidity, malaria still takes a tremendous toll on human health. The disease is caused by five species of the genus *Plasmodium* with *Plasmodium falciparum* as the causative agent of the most virulent form of malaria. In 2015, there were 438,000 deaths from malaria, mostly children in sub-Saharan Africa (WHO, 2015). The symptoms of human malaria are caused by the asexual red blood cell (RBC) stages of the parasite. Within the RBC, the parasite develops in its own compartment surrounded by the parasitophorous vacuolar membrane (PVM). During this symptomatic phase, the intraerythrocytic parasite modifies the host RBC to make it suitable for its survival and growth. The ~ 48 h asexual life cycle of a parasite is complex, with three successive distinct morphological stages (ring, trophozoite and schizont). Ultrastructural studies have indicated that ring stage parasites are cup shaped (Aikawa et al., 1967; Langreth et al., 1978). At this stage, host cell modification is at its peak with the parasite exporting remodeling and virulence proteins into the RBC (Marti et al., 2005). The trophozoite stage, from approximately 24 to 36 h after invasion, is characterised by rapid parasite growth, repeated DNA replication and the appearance of hemozoin (inert remnant of digested hemoglobin) in the food vacuole. Finally, the parasite enters the schizont stage (~36–48 h) during which individual nuclei and associated organelles are partitioned to produce daughter parasites during a specialised type of cytokinesis called schizogony, and generates up to 32 daughter merozoites (Francia and Striepen, 2014). The host cell then ruptures and releases the merozoites which may then go on to invade new RBCs. The parasite, apart from possessing the classically observed organelles of eukaryotic cells, contains some more atypical compartments, probably driven by its need to survive in an unusual biological niche. These include the apicoplast, a four-membrane bounded plastid-like organelle, the food vacuole, a lysosome-like compartment that contains proteolytic enzymes that degrade host cell hemoglobin internalised by the parasite, and finally specialised secretory organelles forming the apical complex (micronemes, rhoptries and dense granules), located at the apical pole of the merozoite and playing critical roles during host cell invasion. Uncovering the principles governing the biogenesis of these various cellular compartments is of great interest because they likely represent sources of new potential targets for the development of antimalarial therapeutic drugs.

Phosphoinositides are phospholipids found on the cytosolic surface of a variety of intracellular membranes in eukaryotic cells. They contain an inositol head group that can be reversibly phosphorylated at three positions. Although they account for less than

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1% of total cellular lipids, they are of paramount significance in a variety of processes such as signal transduction, cell motility, cytoskeletal reorganisation, DNA synthesis, cell cycle, adhesion, membrane transport, permeability and trafficking (Corvera et al., 1999; Di Paolo and De Camilli, 2006; Shewan et al., 2011). Phosphorylation and dephosphorylation of inositol head groups by different kinases and phosphatases result in seven different phosphatidyl inositol phosphate (PIP) species including three phosphatidylinositol monophosphates (PI3P, PI4P and PI5P), three phosphatidylinositol biphosphates (PI(3,4)P2, PI(4,5)P2 and PI(3,5)P2) and one phosphatidylinositol triphosphate (PI(3,4,5)P3 (Vanhaesebroeck et al., 2001). Each of these seven PIPs has a unique subcellular membrane distribution (see below). Furthermore, within a given membrane, the localisation of specific PIPs can be heterogeneous. Altogether, these enzymatic reactions lead to a specific PIP code, where certain subcellular membranes are enriched or depleted of specific PIPs, creating a membrane identity (reviewed in Kutateladze (2010)).

To shed light on the respective roles and localisation of the different species of PIPs, live-cell imaging studies have provided relatively clear maps of the intracellular PIP distribution in several types of eukaryotic cells (reviewed in Kutateladze (2010)) (Simon et al., 2013). PI3P largely resides in early endosomes (Gillooly et al., 2000) and contributes to endosomal maturation, cargo protein degradation and cell signalling (Simonsen et al., 1998; Christoforidis et al., 1999; Jean and Kiger, 2014), and autophagy (Petiot et al., 2000). Some pools of specific PIPs can be found at more than one location in a cell. For example, PI4P is highly enriched at the Golgi membrane where it is involved in trafficking events and a deficiency in PI4P has been shown to affect Golgi structure and function (reviewed in De Matteis et al. (2005)) while another pool is found at the plasma membrane where it acts as a precursor for the synthesis of PI(4,5)P2 (Balla et al., 2005; Várnai and Balla, 2006; Hammond et al., 2009). PI(4,5)P2 exists predominantly on the plasma membrane although it can also be delivered from the Golgi complex by membrane carriers (Stefan et al., 2002; Czech, 2003). PI(4,5)P2 is implicated in a number of cell surface related events such as exocytosis, endocytosis, phagocytosis, cell motility, cell adhesion, microtubule capture, regulation of integral membrane proteins and has a central role in cell signalling through its degradation into inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG) by the action of Phospholipase C (reviewed in Di Paolo and De Camilli, (2006)). Unlike constitutive PIPs, others such as PI5P, PI(3,4)P2 and PI(3,4,5)P3 are rapidly and transiently produced in response to the activation of cell surface receptors and other stimuli. PI(4,5)P2 is the precursor for the synthesis of PI(3,4,5)P3, critical in signalling pathways involved in cell proliferation, migration, chemotaxis, phagocytosis and micropinocytosis (reviewed in Katso et al. (2001); Cantley,(2002)), which, together with PI(3,4)P2, accumulate in the plasma membrane but only after specific signalling activation (McLaughlin and Murray, 2005; Balla, 2013). PI(3,4)P2 is also found at the early endocytic pathway and although poorly characterised, specific roles of PI(3,4)P2 have been described in both clathrin-dependent and independent endocytosis (Posor et al., 2013; Li and Marshall, 2015). There are only small amounts of PI5P in resting cells and so its function remains poorly characterised but after certain types of stimuli, PI5P accumulates at the plasma membrane and in the nucleus and nuclear PI5P has been proposed to act as a stress response element (Gozani et al., 2003; Jones et al., 2006). As for PI(3,5)P2, it is found to be enriched in the late compartments of the endosomes where it may regulate endosomal operations (fission and fusion) that maintain endomembrane homeostasis and proper performance of the trafficking pathways emanating from or traversing endosomes in yeast, mammalian and plant cells (Gary et al., 1998; Rutherford et al., 2006; de Lartigue et al., 2009; van Gisbergen et al., 2012; Zhang et al., 2012).

Despite their central role in the cellular biology of several types of eukaryotic cells, comparatively little is known about the role of PIPs in the malaria parasite *P. falciparum*. Normal mature mammalian RBCs have only small amounts of detectable PIPs, however upon infection with *P. falciparum,* the phosphoinositide profile undergoes profound changes with important increases in PI3P, PI4P and PI(4,5)P2, and the detection of some small amounts of PI(3,4)P2 and PI(3,4,5)P3 (Vial et al., 1990; Tawk et al., 2010). The latter finding is interesting since these two PIP species are usually not detected in other unicellular organisms where only class III PI3-kinases are present (Tawk et al., 2010; Brown and Auger, 2011). What functions the individual PIPs might play has recently become the focus of a number of studies. PI3P is thought to play a role in processes such as hemoglobin uptake to the food vacuole (Elabbadi et al., 1994; McIntosh et al., 2007; Wengelnik and Vial, 2007; Tawk et al., 2010; Vaid et al., 2010), biogenesis of the apicoplast (Tawk et al., 2010, 2011), resistance to artemisinin (Mbengue et al., 2015) and export of proteins to the erythrocyte (Bhattacharjee et al., 2012) although the latter data have recently been challenged (Boddey et al., 2016). Inhibition of a *P. falciparum* PI4kinase with imidazopyrazines and quinoxalines has revealed that PI4P was likely critical for proper plasma membrane ingression during schizogony (McNamara et al., 2013). Much of what is known with regards to PI(4,5)P2 in malaria seems to be related to its involvement in calcium signalling cascades as a substrate for Phospholipase C in processes such as male gametocyte exflagellation (Ogwan'g et al., 1993; Martin et al., 1994), gametocyte activation (Raabe et al., 2011), synchronization of the erythrocytic cycle by the hormone melatonin (Hotta et al., 2000; Beraldo et al., 2007), sporozoite gliding motility (Carey et al., 2014), merozoite egress (Agarwal et al., 2013; Collins et al., 2013) and potentially subsequent erythrocyte invasion (Vaid et al., 2008). Finally, PI(3,5)P2 has not been detected in *P. falciparum*-infected RBCs (iRBCs) (Tawk et al., 2010) and PI5P has also not yet been reported for any *Plasmodium* spp.

In an effort to gain a deeper understanding of the multiple roles that PIPs are likely to play in the *P. falciparum* erythrocytic cycle, we undertook a comprehensive analysis of the subcellular localisation of each individual PIP species throughout the asexual blood stages. Our results show that the distribution of most PIPs is quite dynamic between the different steps of the cycle. We confirm that PI3P is found at the digestive vacuole membrane and the apicoplast and reveal that PI4P is found at the Golgi apparatus and, similar to PI(4,5)P2, at the plasma membrane. Furthermore, we demonstrate the presence of PI5P in *P. falciparum* and show that it localises to the plasma membrane and potentially the transitional endoplasmic reticulum. Our map will be a useful tool in the further unravelling of the numerous roles played by PIPs in the malaria parasite *P. falciparum*.

2. Materials and methods

This study was approved by the Canadian Blood Services (CBS) research ethics board, project number 2015.001 and by the Centre Hospitalier Universtaire (CHU) de Québec institutional research board, Canada, project number 2015–2230, B14-12-2230, SIRUL 104595. Written consent was obtained by the CBS for all study participants.

2.1. Parasite culture

Plasmodium falciparum 3D7 parasites were originally obtained from David Walliker at Edinburgh University, Scotland. *Plasmodium falciparum* asexual stage parasites were cultured under standard conditions in RPMI-HEPES medium at 4% hematocrit

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