Review

Eukaryotic virulence determinants utilize phosphoinositides at the ER and host cell surface

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Similar to bacteria, eukaryotic pathogens may utilize common strategies of pathogenic secretion, because effector proteins from the oomycete *Phytophthora infestans* and virulence determinants from the human malaria parasite *Plasmodium falciparum* share a functionally equivalent host-cell-targeting motif (RxLR-dEER in *P. infestans* and RxLxE/D/Q in *P. falciparum*). Here we summarize recent studies that reveal that the malarial motif may function differently than previously envisioned. Binding of the lipid phosphatidylinositol 3-phosphate [PI(3)P] is a critical step in accessing the host for both pathogens, but occurs in different locations. Nanomolar affinity for PI(3)P by these short amino acid motifs suggests that a newly identified mechanism of phosphoinositide binding that unexpectedly occurs in secretory locations has been exploited for virulence by diverse eukaryotic pathogens.

Eukaryotic pathogens target proteins into plant and human cells

Numerous microbes parasitize the intracellular environment of plants and mammals. To invade and survive in a host cell, prokaryotic and eukaryotic pathogens communicate across several membrane barriers and are able to modulate host endomembrane systems. Consequently, intracellular microbes develop strategies to modulate host membrane trafficking. Phosphoinositides (PIs) are key players in trafficking and signaling processes in eukaryotes that control membrane–cytoskeleton interactions and vesicle trafficking [1]. In particular, for endocytic trafficking, phosphatidylinositol-3-phosphate [PI(3)P] plays a crucial role in conferring identity to endosomes and regulating vesicle fusion on the cytoplasmic face of the membrane [2], and these properties may be exploited by pathogens to establish intracellular infection.

For some bacterial pathogens, manipulation of cytoplasmic PIs and membrane trafficking begins before they become intracellular. Bacteria such as *Salmonella* invade epithelial cells that are not phagocytic. They do so by actively injecting effectors into host cells, which induces phagocytosis in non-phagocytic cells [3]. These effectors are delivered through a bacterial secretion apparatus inserted into the host plasma membrane [4]. Once injected, these effectors modulate functions of small GTPases as well as cytoskeleton and membrane lipids in the cytoplasm of the host cell. The *Salmonella* effector protein SopB is a phosphatase whose activity recruits the host Rab5 and PI(3)P kinase Vps34 to the *Salmonella*-containing vacuole (SCV) [5]. This induces remodeling in the cytoplasmic actin network underneath the plasma membrane and facilitates bacterial engulfment by host cells (Figure 1a). Subsequently, SopB-induced enrichment of PI(3)P contributes to maturation of the SCV. By contrast, intravacuolar *Mycobacterium tuberculosis* uses the phosphatase SapM (secreted acid phosphatase M) to decrease cytoplasmic PI(3)P levels on the vacuole and thus arrests maturation of the vacuole at the early endosome stage in which *M. tuberculosis* is able to replicate [6].

Viruses are not known to utilize cellular PI(3)P, but utilize other PIs. Lipid-enveloped viruses that harbor a lipid membrane bilayer derived from their host cell, such as HIV-1 and human T-lymphotropic virus-1 (HTLV-1), utilize $PI(4,5)P_2$ and mono- and polyvalent PIs enriched on the inner leaflet of the plasma membrane (PM), including phosphatidylinositol 4-phosphate [PI(4)P], phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$], and phosphatidylinositol-3,4,5-trisphosphate [$PI(3,4,5)P_3$], to assemble and exit the cell [7,8]. Furthermore, PI(4)P is required for replication of RNA viruses from the Picornaviridae (poliovirus, coxsackievirus, Aichi virus, and enterovirus 71) and Flaviviridae (hepatitis C virus) families [9], and inhibition of host PI(4) kinases may serve as a mechanism of panviral therapy [10].

By contrast, eukaryotic pathogens, such as the oomycete *Phytophthora infestans* and the apicomplexan *Plasmodium falciparum*, appear to utilize PI(3)P at the host PM and

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Keywords: malaria; oomycetes; host-targeting; phosphoinositides; secretion; pathogenesis.

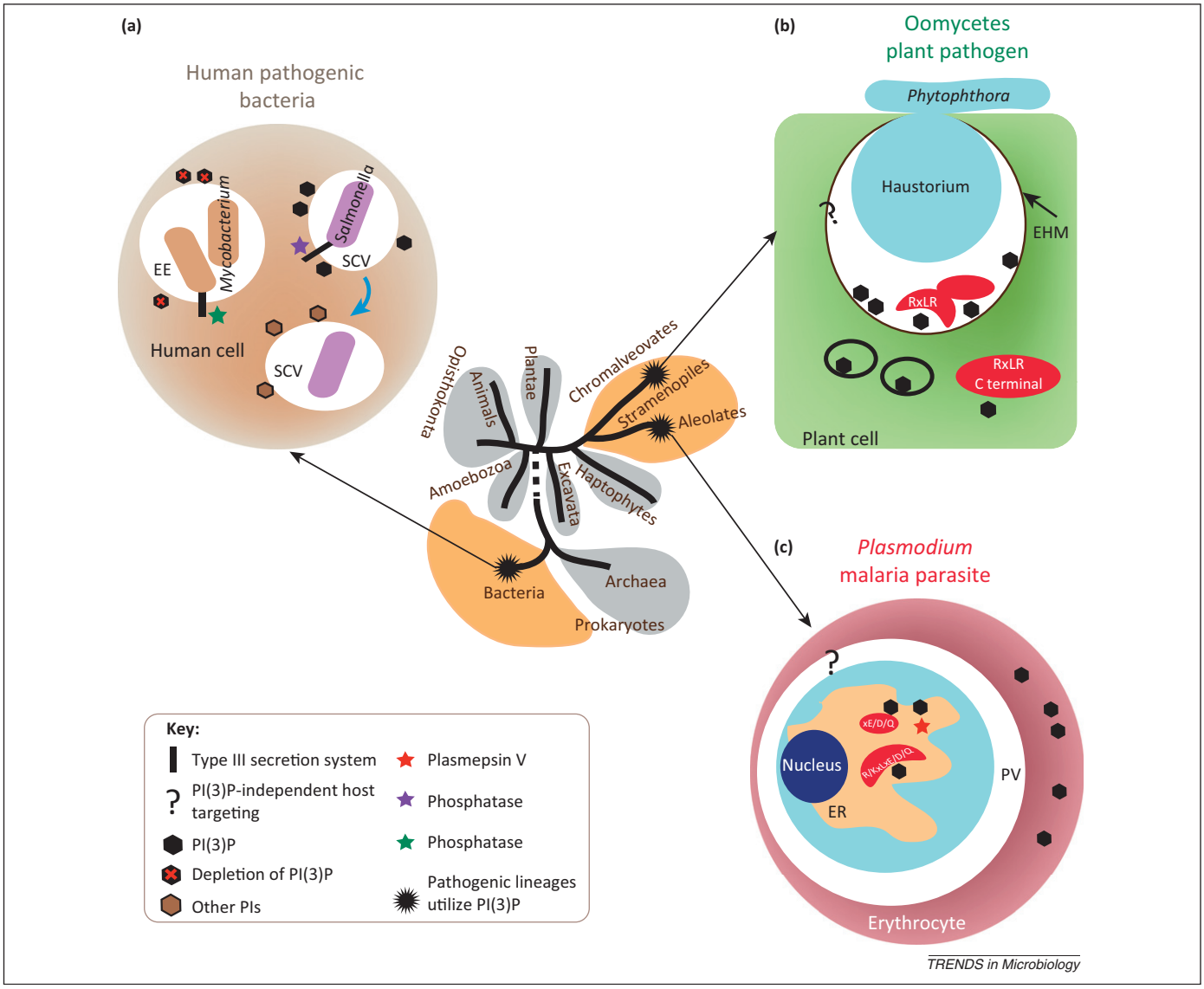

Figure 1. Phylogenetic position, modulation of phosphatidylinositol-3-phosphate [PI(3)P] and host targeting in different intracellular pathogens. The tree of major life domains shown is based on analysis of [50]. Pathogenic lineages that utilize PI(3)P for host targeting and host modulation are chromalveolates, alveolates, and bacteria. The life domains to which they belong are colored yellow. Host targeting refers to the process by which intracellular pathogens deliver pathogen-produced enzymes, toxins, and other proteins directly into the host cytoplasm and membrane. Bacterial pathogens use a type III secretion system to inject effectors into host cells. Eukaryotic pathogens utilize endocytic pathways to deliver proteins. **(a)** Intracellular bacteria use type III effectors to modulate PI(3)P levels for survival and replication within vacuoles inside the host cell cytoplasm. *Mycobacterium tuberculosis* decreases PI(3)P levels to arrest endosome maturation, avoiding destruction by the host. *Salmonella* transiently increases PI(3)P levels to facilitate invasion and biogenesis of the vacuoles for intracellular survival. **(b)** Plant pathogenic oomycetes partly grow into plant cells with a structure called the haustorium. Host targeting utilizes PI(3)P at the extra-haustorial membrane (EHM) surrounding the haustorium to facilitate RxLR-dEER effector entry. How effectors are delivered across the EHM into the host cell is not known (and thus shown by a question mark), but once in the cytoplasm, their C-terminal domain(s) can bind cytoplasmic PI(3)P to modulate host physiology. **(c)** The malaria parasite *Plasmodium* survives in a membranous structure in erythrocytes. Host targeting starts at the parasite ER with binding of PI(3)P by a host-targeting motif R/KxLxE/D/Q. Plasmepsin V is an aspartic protease that does not cleave KxL motifs. However, it is detected in association with PI(3)P (Figure 3) and cleaves after the RxL to yield xE/D/Q, which does not bind PI(3)P but supports export by unknown mechanisms (depicted by a question mark). In oomycetes, PI(3)P-independent host targeting pathways also exist. Vaid *et al.* reported the presence of PI(3)P in infected erythrocytes [51]. Abbreviations: EE, early endosome; SCV, *Salmonella*-containing vacuole; ER, endoplasmic reticulum; PV, parasitophorous vacuole.

the parasite endoplasmic reticulum (ER) lumen to modulate endocytic and exocytotic trafficking pathways, respectively, for both secretion and pathogenesis (Figure 1b,c). *P. infestans* causes potato blight. Infection begins when spores germinate and develop into an infection structure called the appresorium to penetrate plant cells. Subsequently, the pathogen develops a structure called a haustorium for nutrient acquisition and host immunity modulation. The haustorium is surrounded by an extra haustorial membrane (EHM) that separates the pathogen from the plant cytoplasm (Figure 2a). When blood-stage malaria parasites invade human erythrocytes they become enclosed within a parasitophorous vacuolar membrane (PVM) that forms during invasion and encloses the intracellular parasite during the asexual life cycle (Figure 2b). In *P. falciparum*, invasion is completed in minutes but intracellular development in the erythrocyte takes ~48 h. Parasite progeny proliferate within and then rupture out of the PVM and the infected erythrocyte membrane to reinvade new erythrocytes and thus maintain blood-stage infection, which is responsible for all the symptoms and pathologies associated with malaria.