



Subversion of phosphoinositide metabolism by intracellular bacterial pathogens

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Phosphoinositides are short-lived lipids, whose production at specific membrane locations in the cell enables the tightly controlled recruitment or activation of diverse cellular effectors involved in processes such as cell motility or phagocytosis. Bacterial pathogens have evolved molecular mechanisms to subvert phosphoinositide metabolism in host cells, promoting (or blocking) their internalization into target tissues, and/or modifying the maturation fate of their proliferating compartments within the intracellular environment.

Microbial pathogens have developed different strategies to adapt and survive in their specific hosts. Many grow and replicate in the extracellular space in close association with eukaryotic cells, whereas intracellular microorganisms, after uptake in phagocytic or non-phagocytic cells, replicate in different organelles or intracellular compartments where they are protected from antibodies or complement^{1,2}. In all cases, the infection process requires a very precise subversion of the cellular machinery for the parasite to establish an appropriate niche for replication. Exploitation of the cellular cytoskeleton and associated proteins that modulate its plasticity has been reported for many bacterial pathogens^{1,2}. Lipids and lipid metabolism have only recently emerged as additional targets for bacterial virulence factors³. Cellular lipids, and in particular phosphoinositides, not only modulate the function of the actin cytoskeleton or its architecture, but also function as scaffolds recruiting specific effectors to membranes, and/or controlling maturation of intracellular compartments. Modification of phosphoinositides by kinases and phosphatases controls in a very precise way their temporal and spatial distribution (Fig. 1), making them ideal modulators of local and transient cellular mechanisms^{4,5}. In this review, we will address recent data concerning the subversion of phosphoinositide metabolism during bacterial infection.

Phosphoinositides and phagocytosis

Actin rearrangements are required for phagocytosis⁶, and actin remodeling is linked to changes in phosphoinositide metabolism^{7,8}. In particular, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) controls the function and distribution of several actin-binding proteins, through recognition of its headgroup by lysine- or arginine-rich hydrophobic motifs or pleckstrin homology (PH) domains⁹. Other phosphoinositides can be also recognized by PH domains of different specificities or by other domains such as PX or FYVE (Table 1). Their fusion to fluorescent proteins such as green fluorescent protein (GFP) has yielded instrumental probes for the analysis of the distribution of phosphoinositides during phagocytosis and phagosome maturation.

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In macrophages, PtdIns(4,5)P₂ accumulates in pseudopods during extension of the phagocytic cup, and this accumulation coincides with actin enrichment¹⁰, confirming the idea that PtdIns(4,5)P₂ recruits effectors involved in regulating the actin cytoskeleton. One isoform of the type I phosphatidylinositol 4-phosphate 5-kinase (PIP(5)KI α) that synthesizes PtdIns(4,5)P₂ from PtdIns(4)P is also recruited at sites of phagocytosis and is required for completion of the phagocytic process¹¹, suggesting that PtdIns(4,5)P₂ is directly produced at sites of particle internalization (Fig. 2). As the phagosome seals, PtdIns(4,5)P₂ and actin disappear from the phagocytic cup. Catabolism of PtdIns(4,5)P₂ could be explained, in part, by its hydrolysis to diacylglycerol (DAG) by phospholipase C- γ 1 (PLC- γ 1), which is activated during phagocytosis¹². Another fraction of PtdIns(4,5)P₂ is phosphorylated to phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P₃), an event that class I PtdIns 3-kinase (PI(3)K) is responsible for; silencing of the α or β isoforms of p85 — the regulatory subunit of the heterodimeric class I PI(3)K — reduces accumulation of PtdIns(3,4,5)P₃ and blocks phagocytosis¹³. PtdIns(3,4,5)P₃ accumulates at the phagocytic cup¹⁴ and seems to be required for its closure¹⁵ (Fig. 2). Several guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) of the Arf and Rho families of small GTP-binding proteins possess PH domains that interact with PtdIns(3,4,5)P₃ and could be involved in completion of phagocytosis¹⁶. PtdIns(3,4,5)P₃ disappears shortly after sealing of the phagosomal vacuole, and the 5' phosphatase SHIP or the 3' phosphatase PTEN are probably required for dephosphorylating PtdIns(3,4,5)P₃ into PtdIns(3,4)P₂ or PtdIns(4,5)P₂, respectively^{17,18}.

Table 1 Phosphoinositides and phosphoinositides binding domains

Phosphoinositides	Phosphoinositide-binding domain
PtdIns(4)P	PH
PtdIns(5)P	PX ⁷⁷ , PHD ⁷⁸
PtdIns(3,4)P ₂	PH
PtdIns(3,5)P ₂	None identified
PtdIns(4,5)P ₂	PH, ANTH, ENTH, FERM, AP-2 α , tubby
PtdIns(3,4,5)P ₃	PH

(modified from ref. 9)

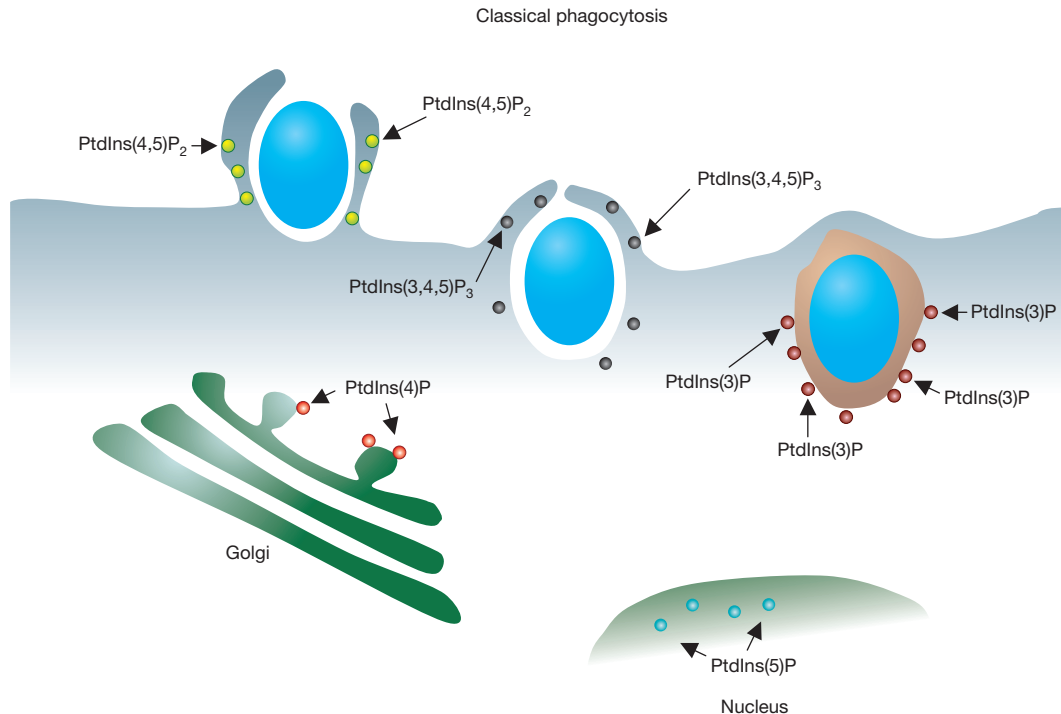


Figure 2 Phosphoinositide species involved in classical phagocytosis. PtdIns(4,5)P₂ accumulates in pseudopods during extension of the phagocytic cup and probably recruits effectors involved in actin cytoskeleton regulation. As the phagosome seals, PtdIns(4,5)P₂ disappears. This could be explained in part by its catabolism by phospholipases but also by its conversion into PtdIns(3,4,5)P₃, and indeed, PtdIns(3,4,5)P₃ appearance coincides with PtdIns(4,5)P₂

clearance. PtdIns(3,4,5)P₃ accumulates transiently in the phagocytic cup and is required for its closure. Once the phagosome is formed, PtdIns(3)P is produced on its surface and recruits proteins that control phagosome fusion and maturation. Other phosphoinositide species are present in the *trans*-Golgi complex (PtdIns(4)P) or in the nucleus (PtdIns(5)P), leading to the proposal that membrane identity can be mediated by compartmentalization of specific phosphoinositides⁷⁹.

tuberculosis and *Legionella pneumophila*). The so-called ‘invasive’ pathogens have evolved mechanisms to induce their own uptake by cells that are normally non-phagocytic, and although some are only transiently present within a vacuole at one stage of their infectious process (for example, uropathogenic *E. coli* (UPEC), or *Yersinia* species), others establish residence in a membrane-bound vacuole in which they replicate (for example, *Salmonella enterica*). Finally, a few invasive bacteria escape from the internalization vacuole and multiply in the cytosol (for example, *Listeria monocytogenes*, *Shigella flexneri* and *Mycobacterium marinum*). The study of phosphoinositide metabolism during bacterial infection reveals that pathogens control these lipids in different ways, exploiting and fostering their normal metabolism, or modifying their cellular signalling cascades to establish their replicative niches (Fig. 3).

Phosphoinositide signalling through receptor engagement

Enteropathogenic *Yersinia* species translocate across the intestinal epithelium in mammalian hosts¹, multiply in local lymph nodes or deep organs and induce systemic diseases²⁶. Interaction between the bacterial outer membrane protein invasins and heterodimeric β1 integrin receptors results in uptake²⁷, a process requiring activation of the small GTP-binding protein Rac1²⁸. The Rac1 pathway involves recruitment of PIP(5)KIα and transient local production of PtdIns(4,5)P₂ at sites of bacterial entry²⁹. Indeed, transfection of Rac1 increases the level of PIP(5)KIα around internalized bacteria. Reduction of PtdIns(4,5)P₂ produced by PIP(5)KIα using a membrane-targeted PtdIns(4,5)P₂ phosphatase lowers bacterial uptake, highlighting the key role of this phosphoinositide in infection. PtdIns(4,5)P₂ production is normally regulated by the GTP-binding protein Arf6 (ref. 30), and expression of a defective Arf6 variant in target cells inhibits PtdIns(4,5)P₂ production and bacte-

rial phagocytosis, but not PIP(5)KIα recruitment²⁹. This observation reveals that wild-type Arf6 is involved in PIP(5)KIα activation to induce PtdIns(4,5)P₂ generation. Together, these results suggest that engagement of β1 integrins by invasins induces the activation of Rac1, which in turn favours the recruitment of PIP(5)KIα at the bacterial entry site; Arf6 then activates PIP(5)KIα, leading to PtdIns(4,5)P₂ formation and translocation to the plasma membrane of PtdIns(4,5)P₂-binding proteins involved in control of actin dynamics required for bacterial invasion. Interestingly, Arf6 exchange factors bind to PtdIns(4,5)P₂ (ref. 31), suggesting that a positive feedback loop could be involved in the activation of Arf6 at sites of high PtdIns(4,5)P₂ concentration. Arf6 has been implicated in the delivery of membranes at sites of phagocytosis³² and this function could also be involved in the formation of the phagocytic cup in which *Yersinia* is internalized. Alternatively, PtdIns(4,5)P₂ could also be recruited to generate PtdIns(3,4,5)P₃, a phosphoinositide species able to recruit other cellular effectors that could regulate actin dynamics during the invasion process (Fig. 3a).

L. monocytogenes is a bacterial pathogen responsible for severe food-borne infections in humans leading to gastroenteritis, meningitis and abortions³³. *L. monocytogenes* induces its internalization in non-phagocytic cells through the interaction of two bacterial invasion proteins, InlA and InlB, with two cellular receptors, the adhesion molecule E-cadherin (which interacts with InlA³⁴) and the hepatocyte growth factor receptor Met (which interacts with InlB³⁵). It is almost ten years since the observation that *L. monocytogenes* entry into target cells requires PI(3)K activity, as bacterial invasion is blocked by the PI(3)K inhibitors wortmannin and LY294002 (ref. 36). In Vero cells, which lack E-cadherin and in which entry is dependent only on the InlB pathway, the use of a dominant-negative form of the p85α regulatory subunit blocked infection,

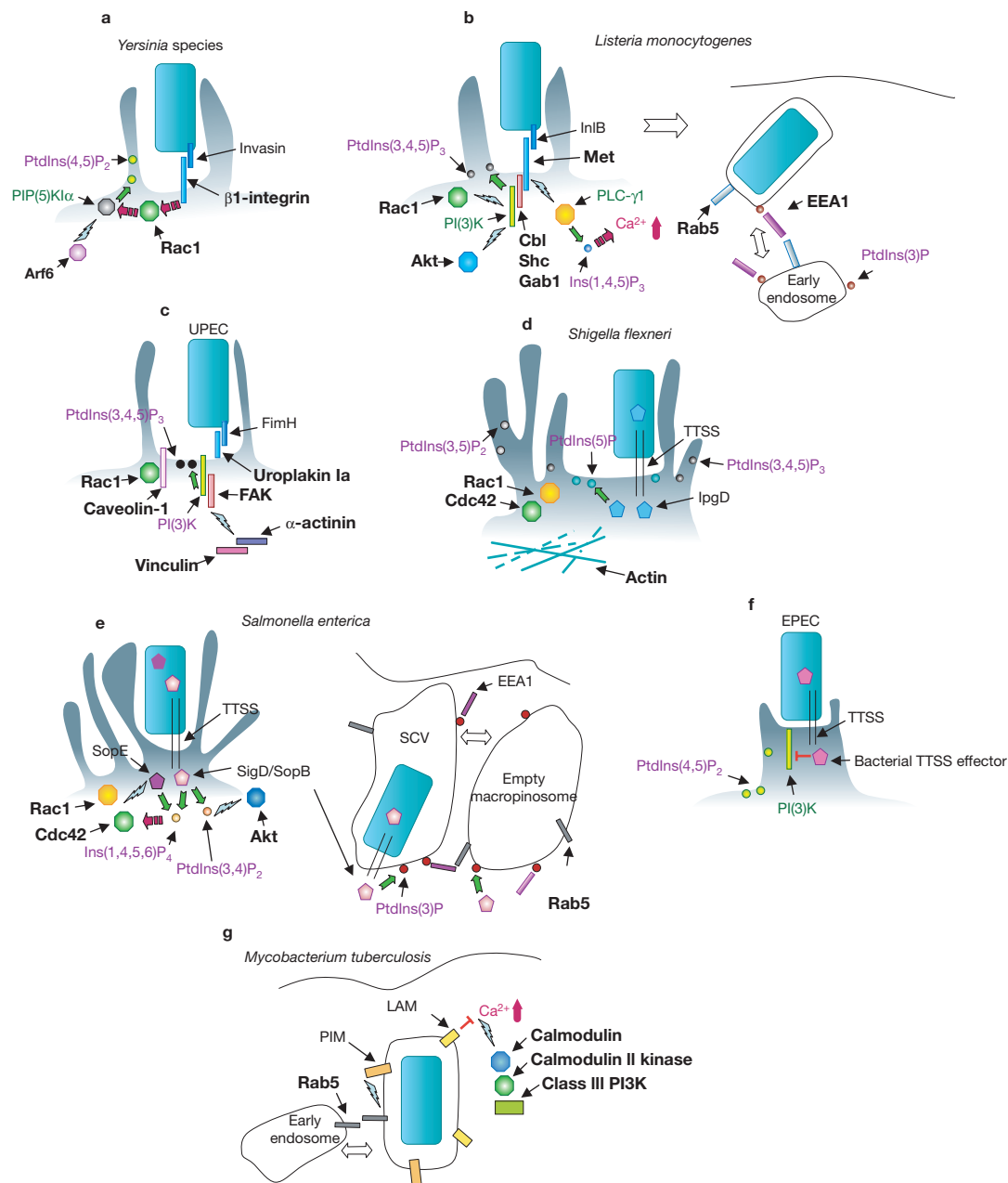


Figure 3 Signalling pathways exploited by bacterial pathogens.

(a) Internalization of *Yersinia* species. Interaction of invasins with $\beta 1$ -integrins induces Rac1 recruitment and PIP(5)K(1) α translocation to the bacterial entry site; Arf6 activates PIP(5)K(1) α leading to local PtdIns(4,5) P_2 generation. (b) Cellular invasion by *L. monocytogenes*. (1) InlB binds to Met, inducing Gab1/Cbl/Shc and class I PI(3)K recruitment; Rac1 is then activated, controlling actin dynamics during entry; Akt is also activated, probably leading to cell survival after infection; PLC- $\gamma 1$ activation induces Ins(1,4,5) P_3 increase and Ca^{2+} release from intracellular stores, but the role of this signalling cascade is unknown. (2) Intracellularly, *L. monocytogenes* resides transiently in an EEA1- and Rab5-enriched vacuole, favouring vacuolar fusion with early endosomes to delay phagosomal maturation. (c) UPEC entry into bladder cells. The FimH receptor is uroplakin Ia, which has a short cytoplasmic domain and interacts with unknown molecules to trigger entry; complexes required for invasion include Rac1/caveolin1 and class I PI(3)K/FAK, the latter probably modulating vinculin and α -actinin association to stabilize actin. (d) Internalization of *S. flexneri*. The TTSS effector IpgD dephosphorylates PtdIns(4,5) P_2 into PtdIns(5)P leading to membrane detachment from the underlying

cytoskeleton, facilitating Rac1 and Cdc42 ruffling activity; PtdIns(3,5) P_2 and PtdIns(3,4,5) P_3 also accumulate at the bacterial entry site, but their possible role in entry is unknown. (e) *S. enterica* invasion of target cells. (1) the Rac1/Cdc42 exchange factor SopE and the phosphatidylinositol phosphatase SigD/SopB promote Ins(1,4,5,6) P_4 generation and indirect Cdc42 activation; SigD/SopB can also generate PtdIns(3,4) P_2 , activating Akt. (2) After invasion, *S. enterica*-containing vacuoles (SCV) are enriched in PtdIns(3)P, favouring SCV fusion with PtdIns(3)P-enriched empty macropinosomes, probably taking advantage of the EEA1 and Rab5 fusogenic machinery. (f) Phagocytosis inhibition by EPEC. Bacteria multiply extracellularly on top of pedestals; translocation to the host cell cytoplasm of an unknown TTSS effector leads to inactivation of class I PI(3)K, PtdIns(4,5) P_2 accumulation and blockage of bacterial internalization. (g) Internalization of *M. tuberculosis*. The bacterial phosphatidylinositol analogue LAM inhibits intracellular Ca^{2+} rise, blocking a calmodulin-calmodulin kinase II pathway that activates class III PI(3)K; simultaneously, the mycobacterial phosphoinositide PIM activates a GTPase (probably Rab5) inducing fusion of the *M. tuberculosis*-containing compartment with early endosomes.

revealing a role for the class I PI(3)K in this process. *L. monocytogenes* takes advantage of the cellular signalling pathway associated with the tyrosine kinase receptor Met to recruit adaptors such as Gab1, Shc and Cbl, which translocate the class I PI(3)K from the cytosol to the plasma membrane to induce cytoskeletal rearrangements³⁷. Interestingly, the purified InlB protein is capable of inducing membrane ruffles in target cells as HGF, the natural ligand of Met^{37,38}. The activation kinetics of Met by HGF and InlB are different³⁵, but the signalling cascades triggered by both molecules seem similar^{39,40} (Fig. 3b). The direct downstream effectors that could be recruited by PtdIns(3,4,5)P₃ are, however, unknown. Invasion of Vero cells by *L. monocytogenes* requires the polymerization and subsequent depolymerization of actin from the phagocytic cup, a process that is dependent on the actin-depolymerizing factor cofilin, a downstream effector of Rac⁴⁰. The Rac exchange factor Vav2, a PH-domain protein that binds to PtdIns(3,4,5)P₃, was considered as a potential downstream PI(3)K effector; however, dominant-negative Vav2 does not block *L. monocytogenes* entry (H. Bierne, P. Mandin and P.C., unpublished observations).

In the Caco-2 cell line, which is dependent on the InlA-invasion pathway, wortmannin also impairs bacterial entry³⁶, but the mechanism of class I PI(3)K recruitment by E-cadherin is unknown. It has recently been found that E-cadherin localizes in the plasma membrane within lipid rafts, which are lipidic platforms that select molecular effectors into functional units required for efficient signalling and sorting processes⁴¹. *L. monocytogenes* could take advantage of this platform to recruit signalling molecules involved in translocation of the PI(3)K into the plasma membrane.

In the human epithelial cell line Hep-2, InlB activates a different isoform of class I PI(3)K (p85β-p110) and subsequently activates PLC-γ1, leading to a transient increase in intracellular inositol 1,4,5-triphosphate (Ins(1,4,5)P₃) and release of intracellular calcium⁴². However, pharmacological or genetic inactivation of PLC-γ1 does not affect the InlB-mediated bacterial uptake, indicating that PLC-γ1 activation could be a side effect during the entry process; alternatively, PLC-γ1 activation could function in post-internalization steps, but this possibility has not been explored. After invasion, *L. monocytogenes* resides transiently in a vacuole that is enriched in EEA1, suggesting the presence of PtdIns(3)P in this compartment (Fig. 3b). Interestingly, *L. monocytogenes*-containing phagosomes are also enriched in the small GTPase Rab5, which increases the fusion of early endosomal compartments with the bacterial vacuole, delaying its maturation⁴³. As a consequence, *L. monocytogenes* resides as long as possible in a safe environment before lysing its phagosome and escaping into the cellular cytoplasm where the bacteria multiply. Regulation of PtdIns(3)P levels by *L. monocytogenes* is an attractive hypothesis that could explain the retention of EEA1 and Rab5 in the bacterial phagosome. Activation of the anti-apoptotic Akt kinase downstream of the class I PI(3)K has been detected in the murine cell line J774 (ref. 44), suggesting that *L. monocytogenes* could also modulate cell survival after infection (Fig. 3b).

More than 80% of urinary tract infections are produced by uropathogenic *E. coli* (UPEC). Although UPEC was long considered non invasive, recent results indicate that the type 1 pilus adhesin FimH mediates not only bacterial adherence but also invasion of human bladder epithelial cells⁴⁵. The only known cellular receptor for FimH is uroplakin Ia⁴⁶, a molecule that localizes to lipid rafts; caveolin-1 and Rac1 interact with each other within lipid rafts and are also required for UPEC entry⁴⁷. Wortmannin inhibits invasion, implying that PI(3)K is required for uptake. Phosphorylation of the focal adhesion kinase (FAK) and formation of FAK-PI(3)K complexes are observed only with wild-type UPEC, and not with a FimH mutant⁴⁵. In the current scenario, during bladder cell invasion, UPEC probably take advantage of the physiological

endocytosis of uroplakin Ia, which interacts with caveolin-1, Rac1 and possibly other uroplakins within the context of a lipid raft to trigger internalization signalling cascades requiring PI(3)K activity; PI(3)K and FAK could potentially modulate, directly or indirectly, a number of other molecules including vinculin, tensin, talin or α-actinin, which can function in stabilizing the host-cell cytoskeleton. Indeed, the infection of bladder cells with wild-type UPEC induces the formation of vinculin-α-actinin complexes, and the formation of these complexes is inhibited in the presence of PI(3)K inhibitors⁴⁵ (Fig. 3c).

Translocation of bacterial phosphoinositide phosphatases

S. flexneri is the causative agent of bacillary dysentery in humans⁴⁸. This pathogen induces its entry into non-phagocytic cells by injecting bacterial effectors directly into the host cell through a type III secretion system (TTSS). The TTSS is a needle-like structure that spans the inner and outer membranes of the bacterial envelope and after contact with mammalian cells forms a pore in the host-cell membrane; through this channel bacterial effector proteins are translocated into the host-cell cytoplasm where they subvert cellular functions⁴⁹.

Some of the *S. flexneri* TTSS effectors, such as IpaC, directly interact with cytoskeletal proteins^{1,50,51}. *S. flexneri* also modifies the phosphatidylinositol metabolism of host cells to favour its internalization through the activity of another TTSS effector, the virulence factor IpgD. During infection of non-phagocytic HeLa cells, *S. flexneri* translocates IpgD into the cytoplasm of target cells, which behaves as a phosphatidylinositol 4-phosphatase that specifically dephosphorylates PtdIns(4,5)P₂ into PtdIns(5)P, leading to the accumulation of PtdIns(5)P in the eukaryotic cell⁵². PtdIns(5)P is a phosphoinositide species that was thought not to exist *in vivo*⁵³. Whether PtdIns(5)P has a role in regulating specific cytoskeletal rearrangements is still unknown. However, dephosphorylation of PtdIns(4,5)P₂ has an important role during invasion. PtdIns(4,5)P₂ regulates membrane adhesion by controlling local binding interactions between the plasma membrane and the cytoskeleton⁵⁴; accordingly, in cells transfected with IpgD, the force that tethers the plasma membrane and the cytoskeleton was reduced⁵². Moreover, IpgD transfection led to the formation of membrane blebbing and the disappearance of actin stress fibres. IpgD probably allows the local detachment of the plasma membrane from the cytoskeleton to facilitate extension of membrane filopodia and ruffles by changing the interaction between PtdIns(4,5)P₂ and cytoskeletal anchoring proteins and/or by regulating actin dynamics through enzymes such as gelsolin or cofilin that increase or decrease actin polymerization (Fig. 3d). Cdc42 and Rac favour the extension of membrane filopodia and ruffles during *S. flexneri* invasion, and IpgD seems to facilitate the cytoskeletal modifications induced by these small Rho family GTPases⁵². The specificity of the IpgD inositol 4-phosphatase activity is revealed by the fact that, during infection, only PtdIns(4,5)P₂ levels decrease whereas other phosphoinositide species such as PtdIns(3,4)P₂, PtdIns(3,5)P₂ and PtdIns(3,4,5)P₃ increase, due to concomitant activation of PI(3)K. PI(3)K activity is linked to infection, as PtdIns(3,4)P₂, PtdIns(3,5)P₂ and PtdIns(3,4,5)P₃ concentrations do not increase with non-invasive mutants⁵²; wortmannin does not block entry, suggesting either that PI(3)K kinase activity is not necessary for invasion, or that wortmannin-insensitive class II enzymes or a bacterially derived kinase are involved in this process.

S. enterica is a main cause of food-borne gastroenteritis, as well as the etiological agent of typhoid fever⁵⁵, inducing its entry into non-phagocytic cells and its survival in macrophages, through the action of two different TTSS¹. The *S. enterica* TTSS effector SigD/SopB is an IpgD homologue that functions in the invasion of target cells as well as in the maturation of *S. enterica*-containing vacuoles (SCVs). It presents phosphatidylinositol polyphosphate and inositol polyphosphate phosphatase activities; however, there are conflicting reports about its enzymatic specificity^{56–58} (Fig. 1).

SigD/SopB stimulates the formation of membrane ruffles and Cdc42-dependent actin cytoskeletal rearrangements required for *S. enterica* entry in Henle-407 cells⁵⁷. This function overlaps with the activity of SopE and SopE2, two other TTSS effectors that function as exchange factors for Cdc42 and Rac. SigD/SopB dephosphorylates inositol-1,3,4,5,6-phosphate (Ins(1,3,4,5,6)P₅) into inositol-1,4,5,6-phosphate (Ins(1,4,5,6)P₄) in infected cells as well as in *in vitro* assays⁵⁷. SopE does not have inherent inositol phosphate phosphatase activity but it does contribute to the hydrolysis of Ins(1,3,4,5,6)P₅ and inositol-1,2,3,4,5,6-phosphate (Ins(1,2,3,4,5,6)P₆) probably by activation of a PLC as well as of an endogenous phosphatase. The molecular pathway that leads to the indirect activation of Cdc42 by inositol phosphates is unknown; these molecules probably compete with phosphoinositides for binding to specific PH domains, displacing the subcellular localization of PH-domain-containing molecules required for actin cytoskeleton rearrangements during invasion⁵⁷ (Fig. 3e).

As stated above, SigD/SopB also has phosphatidylinositol phosphatase activity, and has been implicated in PtdIns(4,5)P₂ turnover during *S. enterica* invasion⁵⁸ (the precise site of PtdIns(4,5)P₂ hydrolysis remains to be determined). SigD/SopB is responsible for the rapid disappearance of PtdIns(4,5)P₂ from the plasma membrane before fission and formation of the SCV⁵⁸. Thus, it has been proposed that SigD/SopB weakens membrane–cytoskeleton interactions, probably by displacing molecules that recognize PtdIns(4,5)P₂, promoting the separation and sealing of the SCV after invasion.

Phagosomal maturation is also controlled by the phosphatidylinositol phosphatase activity of SigD/SopB. In MDCK cells, PtdIns(3)P is produced periodically on the membranes of nascent SCVs in a wortmannin-insensitive manner, and it was suspected that SigD/SopB participated in this process by dephosphorylating PtdIns(3,5)P₂ to PtdIns(3)P⁵⁹. In HeLa cells, PtdIns(3)P also transiently accumulates on SCVs, but this phenomenon is wortmannin-sensitive, implying that a PI(3)K is involved in maturation of the SCV⁶⁰. More recently, it has been observed that PtdIns(3)P cycles on latex-bead phagosomal membranes are dependent on the host cell and are cell type-specific⁶¹. Nevertheless, it has been confirmed in Henle-407 cells that SigD/SopB contributes to SCV maturation, inducing the formation of large PtdIns(3)-enriched macropinosomes during *S. enterica* invasion⁶². The appearance of PtdIns(3)P-enriched empty vesicles that fuse frequently with the SCV suggests that *S. enterica* induces the homotypic fusion of PtdIns(3)P compartments; the PtdIns(3)P-binding effectors EEA1 and Rab5 have been localized to the early SCV⁶³ indicating that *S. enterica* could boost the early endosomal fusion machinery to divert SCV maturation from the normal phagolysosomal pathway (Fig. 3e).

Finally, SigD/SopB is also required for Akt activation in HeLa cells⁶⁴. Cell infection with wild-type *S. enterica* induces the translocation of Akt to the plasma membrane and its phosphorylation on residues Thr 308 and Ser 473. Infection with a SigD/SopB deletion mutant does not induce Akt phosphorylation, although it is translocated to the plasma membrane⁶⁴. Complementation with a SigD/SopB mutant that lacks inositol phosphatase activity does not restore Akt activation, highlighting the relevance of SigD/SopB enzymatic activity during Akt activation. It has been proposed that PtdIns(3,4)P₂, produced from PtdIns(3,4,5)P₃ by SigD/SopB (Fig. 1) could induce a conformational change in Akt favouring its phosphorylation and full activation leading to increased survival of infected epithelial cells⁶⁵.

Bacterial interference with phosphoinositide kinases

Enteropathogenic *E. coli* (EPEC), a major cause of severe infantile diarrhoea, colonizes the human small intestine mucosa and exerts its pathological effects by inducing the degeneration of the enterocyte brush

border⁶⁶. Through a TTSS, adhering EPEC directly translocate bacterial effectors into the host-cell cytoplasm. These effectors induce the localized loss of microvilli and the formation of actin-rich protrusions called ‘pedestals’, on which bacteria reside⁶⁷. EPEC multiply in the extracellular space and pedestal formation has been considered as a mechanism to block bacterial phagocytosis. Along this line, it has been shown that EPEC inhibit their internalization into host cells through the inhibition of PI(3)K-dependent pathways⁶⁸. In macrophages, an EPEC TTSS mutant can be efficiently phagocytosed whereas a wild-type EPEC cannot. Moreover, chemical inhibition of PI(3)K by wortmannin, or functional inhibition of class I PI(3)K with a dominant-negative p85 α subunit, blocked the internalization of the EPEC mutant. Wild-type EPEC do not inhibit recruitment but do inhibit the activity of PI(3)K, causing the dissociation of phosphotyrosine proteins associated with PI(3)K and inducing interruption of phagocytic signals (Fig. 3f). The accumulation of PtdIns(4,5)P₂ at sites of bacterial adhesion in macrophages suggests that wild-type EPEC also mediate the dephosphorylation of PtdIns(3,4,5)P₃ into PtdIns(4,5)P₂ by an as yet uncharacterized phosphatase.

M. tuberculosis is the etiological agent of tuberculosis, a severe pulmonary disease characterized by the infection and chronic survival of the tubercle bacillus in host macrophages⁶⁹. *M. tuberculosis* resides in a vacuole that remains in an early phagosomal stage characterized by fewer or less active vacuolar H⁺-ATPases⁷⁰ and absence of mature lysosomal hydrolases⁷¹. As shown recently, the bacterial phosphatidylinositol analogue lipoarabinomannan (LAM) inhibits cytosolic calcium rise in infected cells, blocking a calmodulin–calmodulin kinase II cascade that is required for activation of class III PI(3)K, PtdIns(3)P generation and EEA1 recruitment to phagosomes⁷². EEA1, in cooperation with the *trans*-Golgi network (TGN) molecule syntaxin 6, is needed for the delivery of hydrolases from the TGN to the phagosome, and *M. tuberculosis* interferes with this pathway⁷³. It has been recently reported that the PtdIns(3)P ligand Hrs is not present in *M. tuberculosis* phagosomes⁷⁴; its absence is possibly related to the maturation arrest of this compartment. In parallel, another secreted mycobacterial phosphoinositide (phosphatidylinositol mannoside, PIM) stimulates early endosomal fusion (probably influencing Rab5 activity), functioning in concert with LAM to block phagosomal maturation⁷⁵ (Fig. 3g).

It was recently appreciated that, during phagocytosis of latex beads, there are two phases of PtdIns(3)P acquisition: phase I consists of a strong burst of PtdIns(3)P after closure of the phagocytic cup and is calmodulin–calmodulin kinase II-independent, whereas phase II consists of multiple waves of lower PtdIns(3)P peaks and is calmodulin–calmodulin kinase II-dependent⁶¹. These oscillations are proposed to dictate orderly interactions with other compartments involved in phagosomal maturation⁶¹. During invasion of target cells by *M. tuberculosis*, phase I peak (dependent on the presence of cholesterol at the bacterial entry site) is shifted to earlier time points when bacteria are still entering the host cell and the phagosomal cup is not closed, whereas phase II peaks are not observed in mycobacterial phagosomes⁶¹. The absence of phase II peaks is readily explained by the inhibition of class III PI(3)K recruitment by mycobacterial LAM⁷². The shift in peak I could be explained by an early activation of Rab5 by PIM⁷⁵, rendering the *M. tuberculosis* phagosome unique from the early stages of its formation, favouring its divergence from the normal phagolysosomal maturation cascade.

The idea that phosphoinositides could be used as targets for control of infectious diseases is illustrated in an interesting report⁷⁶ that suggests that treating cells infected with *M. tuberculosis* with different lipids derived from phosphoinositide metabolism (such as arachidonic acid, ceramide or sphingosine) could alter the maturation fate of *M. tuberculosis*-containing phagosomes increasing their fusion with

lysosomes, lowering their pH and resulting in a marked decrease in pathogen viability. These results may lead to the potential dietary use of phosphoinositide derivatives to control pathogen replication.

Conclusions

Phosphoinositides have an essential function in the coordination of actin cytoskeleton rearrangements and in phagosomal maturation steps during classical phagocytosis. Bacterial pathogens have taken advantage of the scaffolding role of phosphoinositides to recruit at the bacterial entry site effectors required for promoting (or inhibiting) invasion, or to modify the maturation of their intracellular compartments. Many of the specific effectors controlled by each bacterial pathogen through the modulation of phosphoinositide metabolism remain uncharacterized. We believe that this field will expand rapidly and that the study of bacterial intracellular parasitism will probably shed light onto the mechanisms used by the cell to proceed during classical phagocytosis.

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