Phosphoinositides and host-pathogen interactions.

Javier Pizarro-Cerdá, Andreas Kühbacher, Pascale Cossart

Phosphoinositides and Host-Pathogen Interations

Javier Pizarro-Cerdá[1,2,3], Andreas Kühbacher[4] & Pascale Cossart[1,2,3]

Institut Pasteur, Unité des Interactions Bactéries-Cellules, Paris F-75015, France[1];

INSERM, U604, Paris F-75015, France[2]; INRA, USC2020, Paris F-75015, France[3];

Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Department of Molecular Biotechnology, Stuttgart G-70569, Germany[4]

ABSTRACT

Phosphoinositides control key cellular processes including vesicular trafficking and actin polymerization. Intracellular bacterial pathogens manipulate phosphoinositide metabolism in order to promote their uptake by target cells and to direct in some cases the biogenesis of their replication compartments. In this chapter, we review the molecular strategies that major pathogens including *Listeria, Mycobacterium*, *Shigella*, *Salmonella*, *Legionella* and *Yersinia* use to hijack phosphoinositides during infection.

INTRODUCTION

Of an estimated 58.8 million annual deaths worldwide, approximately 15 millions are believed to be caused by infectious diseases [1], and bacterial pathogens contribute significantly to these numbers. Pathogens such as *Mycobacterium tuberculosis* are passively phagocytosed by macrophages, but others including *Listeria monocytogenes*, *Shigella spp.* or *Salmonella enterica* actively promote their internalization within epithelial cells by hijacking specific host signaling cascades that lead to pathogen engulfment via actin cytoskeleton polymerization and plasma membrane rearrangements (Figure 1) [2]. After host cell entry, bacterial pathogens may occupy different intracellular compartments (Figure 1): *Listeria* and *Shigella* disrupt their internalization vacuole and multiply in the cytoplasmic space; the proliferation of *Mycobacterium*, *Salmonella* and *Legionella pneumophila* mostly occurs in early phagosomal-, late endosomal- and endoplasmic reticulum-related compartments, respectively; however, recent reports indicate that these three pathogens may also reach the cytoplasmic space after rupture of their membrane-bound replication compartments. As reviewed in the chapters of this issue, phosphoinositides play key roles in the regulation of membrane trafficking events as well as in the tight control of actin polymerization, and therefore their metabolism is subverted by intracellular bacterial pathogens to favor cellular invasion or to modify the properties of their replication compartments [3,4]. Regulation of phosphatidylinositol (PtdIns)(4,5)P_2 and PtdIns(3,4,5)P_3 levels at the plasma membrane plays a critical role for the entry of some bacterial pathogens in host cells: while PtdIns(3,4,5)P_3 positively stimulates actin rearrangements at bacterial entry sites, some pathogens lower interactions between the plasma membrane and the actin cytoskeleton by inducing a decrease in PtdIns(4,5)P_2 levels, favoring plasma membrane detachment from the underlying cortical actin cytoskeleton and bacterial engulfment in massive macropinocytic structures. PtdIns(3)P levels are also tightly controlled at intracellular stages of bacterial infection: some pathogens hamper PtdIns(3)P production or function on their replicating compartments to block their fusion with lysosomes, while other pathogens use the fusogenic properties of PtdIns(3)P to remodel their vacuoles. Strikingly, it is through the study of their role in controlling entry or survival of bacterial pathogens in host cells that the functions of PtdIns(4)P and PtdIns(5)P as *bona fide* second messengers have been recently expanded. Here we discuss each specific situation for several well studied pathogens.

Listeria monocytogenes

The Gram positive bacterium *Listeria monocytogenes*, one of the ten species of the genus *Listeria*, is the agent of a food-borne disease that may lead to meningitis in immunocompromised individuals, and abortions in pregnant women. *Listeria* is able to traverse the intestinal, blood-brain and fœto-placental barriers by engaging host cell receptors with bacterial surface proteins and promoting bacterial intracellular uptake in target tissues [5-7].

Two major invasion signaling cascades have been described for *Listeria*: the interaction of InlA with its receptor E-cadherin leads to bacterial internalization within polarized epithelial cells, while interaction of InlB with the hepatocyte growth factor receptor Met leads to bacterial entry within many non-polarized epithelial cells *in vitro* [8,9]. InlB interaction with Met, which is a tyrosine kinase receptor, leads to its auto-phosphorylation and to the recruitment of the protein adaptors Gab1, Shc, Cbl and CrkII which contribute to the translocation/activation of the class IA PI 3-kinase to *L. monocytogenes* entry foci [10-12]. PtdIns(3,4,5)P_3 produced by the class IA PI 3-kinase is a major effector of actin rearrangements in eukaryotic cells [13] and during *Listeria* infection, the distribution of PtdIns(3,4,5)P_3 within cholesterol-rich membrane micro-domains is critical for activation of Rac1 in Vero cells [14] and of the WASP-related complexes WAVE1 and WAVE2, leading to actin polymerization by the Arp2/3 complex [15,16]. A recent RNA interference (RNAi)-based screen identified several PtdIns(3,4,5)P_3-binding molecules which modulate *Listeria* entry including the regulators of small GTPases of the Arf family ARAP2, GIT1 and ARNO or the Rac1 activator SWAP70 [17]; the timing and specific contribution of these molecules to the bacterial entry process remains to be precisely established.

PtdIns(4)P produced by PI 4-kinases is another major cellular phosphoinositide which has been traditionally regarded as a signaling molecule of the Golgi, or as a precursor of PtdIns(4,5)P_2 at the plasma membrane [18]. We have shown that the activity of a class II PI 4-Kα (and to lesser extent of a β isoform) is required for *Listeria* cell invasion downstream of the tetraspanin molecule CD81 in a pathway independent of the PI 3-K pathway [19,20]: indeed, inactivation of class II PI 4-Kα impairs bacterial entry but does not perturb plasma membrane PtdIns(4,5)P_2 or PtdIns(3,4,5)P_3 levels, suggesting that PtdIns(4)P could play a signaling role on its own at the plasma membrane of host cells. Strikingly, the development of a novel PtdIns(4)P biosensor using the PtdIns(4)P-binding module of the protein SidM from *Legionella pneumophila* confirms that this phosphoinositide is present on multiple pools not only at the Golgi but also at the plasma membrane and in late endosomes/lysosomes, confirming our previous observations and suggesting a broader function of PtdIns(4)P as a scaffold molecule in mammalian cells [21,22].

The host protein OCRL is a 5-phosphatase which preferentially dephosphorylates PtdIns(4,5)P_2 and also PtdIns(3,4,5)P_3 [23]. It possesses a clathrin-binding domain, participates in early steps of the endocytic pathway [24], and promotes actin depolymerization required for successful cytokinesis [25,26]. We found recently that *Listeria* invasion is controlled by OCRL, precisely by down-regulating the cellular levels of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 and limiting actin polymerization at bacterial entry sites [27]. Knock down of OCRL by siRNA increases entry. As detected by correlative light-electron microscopy, OCRL and clathrin coated vesicles colocalize around *Listeria*-containing vacuoles (Figure 2), suggesting a functional interaction between clathrin and OCRL during *Listeria* infection.

Interestingly, *Listeria* expresses several enzymes which display a lipid phosphatase activity and are critical for bacterial survival. LipA, in addition to its tyrosine phosphatase activity, dephosphorylates PtdIns(3)P, PtdIns(5)P and PtdIns(3,5)P_2 *in vitro*, and bacteria lacking this effector are severely attenuated in virulence *in vivo* [28], but the cellular functions affected by LipA have not been identified yet. As shown below, *Mycobacterium* also expresses a dual protein/lipid

phosphatase, MptpB, which is an homologue of LipA [29]. PlcA and PlcB are two phospholipases known to be involved in *Listeria* escape from vacuoles: PlcA cleaves phosphatidylinositol and glycosylphosphatidylinositol anchors [30] while PlcB expresses lecithinase activity and hydrolyzes phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin but not phosphatidylinositol [31]. Recently, it has been proposed that these phospholipases, particularly the phosphatidylinositol specific PlcA prevents pre-autophagosomal maturation by reducing the cellular PI concentration and therefore PtdIns(3)P levels. This mechanism was further shown to protect cytoplasmic *Listeria* from autophagy, induced by LLO-dependent amino acid starvation [32].

Following escape from the internalization vacuole, *Listeria* proliferates in the host cell cytoplasm where the bacterial surface protein ActA activates the Arp2/3 complex to promote actin polymerization and bacterial motility [33,34]. It has been proposed that PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 originating from the inner leaflet of the plasma membrane may associate with cytoplasmic *Listeria* and promote actin-based motility [35]. However, the signaling cascades triggered downstream of these phosphoinositides are unknown.

In summary, bacterial manipulation of the host phosphoinositide metabolism, and in particular of class IA PI 3-kinases is critical for actin polymerization and successful *Listeria* invasion of host cells. Regulation of cytoskeletal rearrangements during infection is tightly regulated: actin polymerization is initially required to remodel the plasma membrane around invading bacteria, but actin depolymerization is also necessary to disassemble the actin-rich meshwork underlying the phagocytic cup to complete the invasion process [15]; sustained local actin depolymerization by the host phosphatase OCRL, which down-regulates PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 levels produced at the bacterial entry foci, limits infection [27]. *Listeria* secretes several lipid phosphatases which modulate bacterial vacuolar escape and other steps of the infection cycle [28,32].

Shigella spp.

The genus *Shigella* spp. harbors 4 species or groups (Group A: *S. dysenteriae,* Group B: *S. flexneri*, Group C: *S. doydii*, and Group D: *S. sonnei*). *S. dysenteriae* and *S. flexneri* are the etiological agents of bacillary dysentery, a severe bloody diarrheal disease prevalent in developing countries [36]. A type III Secretion System (T3SS) encoded by a large virulence plasmid allows *Shigella* spp. to inject virulence factors into host colonic epithelial cells, leading to bacterial invasion of the epithelial lining, associated with the elicitation of an intense inflammatory response leading to the destruction of the mucosa [37].

Following *Shigella* capture by filopodia [38], the T3SS effectors IpaB and IpaC form a translocon within the plasma membrane of host epithelial cells which is required for the injection of additional T3SS effectors into the cytoplasm of epithelial cells [39,40]. Among them, IpgD is a PI 4-phosphatase that de-phosphorylates PtdIns(4,5)P_2 into PtdIns(5)P, thus decreasing the membrane tether forces and favoring membrane blebbing at bacterial entry sites [41]. IpaC also fosters the formation of membrane ruffles through activation of the Src tyrosine kinase [42], leading to the Crk-

dependent recruitment of the Src substrate cortactin [43]. Interestingly, *Shigella* is known to interact with T cells and decrease in membrane tether forces by IpgD has also been proposed to inhibit T cell migration [44].

Upon invasion, *Shigella* disrupts its internalization vacuole and proliferates in the host cell cytoplasm. Prolonged survival of infected cells and efficient bacterial replication is sustained by the biological activities of PtdIns(5)P produced by IpgD during the early invasion stages: using the probe GFP-PHDx3, it has been shown by microscopy that PtdIns(5)P concentrates at bacterial entry sites and endosomes, triggering a class IA PI 3-kinase/Akt pathway that leads to phosphorylation of the anti-apoptotic effectors FKHR and GSK3, favoring infected cell survival [45]. PtdIns(5)P also modifies vesicular trafficking and prevents EGFR degradation in late endocytic compartments, prolonging EGFR signaling and survival of *Shigella* infected cells [46]. Finally, production of PtdIns(5)P prevents ATP release from infected cells through hemichannels of infected cells, thereby dampening inflammatory responses in the intestine, and prolonging bacterial survival [47].

Mycobacterium tuberculosis

Mycobacterium tuberculosis is the agent of a severe pulmonary disease characterized by bacterial chronic survival in macrophages [48]. Internalization within professional phagocytes is mediated for the most part by complement receptors and complement opsonization of mycobacteria [49]; the presence of plasma membrane cholesterol-rich micro-domains has been reported to be critical for the entry step [50]. *Mycobacterium* survival in host cells has been traditionally associated to proliferation within a phagosome that fails to acidify [51] and which is enriched in the early endosomal small GTPase Rab5 but is devoid of the late endosomal Rab7 [52]. Recent reports indicate that *Mycobacterium* is actually able to translocate to the cytosol of infected cells [53] resulting in toxicity and cell death [54]. Crosstalk between *Mycobacterium* and the phosphoinositide metabolism has been until now reported essentially for the replicative early phagosomal stages.

Phagosomal maturation arrest at an early stage is in part favored by the bacterial phosphatidylinositol mannoside (PIM), which is actively released from internalized *Mycobacterium* and integrates into phagosomal membranes [55], promoting homotypic fusion of early phagosomes in a Rab 5-dependent and PI 3-kinase-independent manner [56]. However, a major proposed mechanism for phagosomal remodeling involves the down-regulation of PtdIns(3)P levels from mycobacterial-containing compartments [57], leading to the exclusion of key fusion-promoting molecules such as EEA1 or Hrs [58,59], which bind PtdIns(3)P through their FYVE domains and link type III PI 3-kinase activity to endosomal/phagosomal maturation [60-62]. Two pathways have been reported to down-regulate PtdIns(3)P levels on *Mycobacterium*-containing phagosomes: blocking type III PI 3-kinase hVPS34 activity is achieved by the mannose-capped lipoarabinomanan (ManLAM), another mycobacterial phosphatidylinositol analogue which, through a still non-identified mechanism, interferes with the increase of intracellular Ca^{2+} and hampers a phagosomal maturation cascade involving the Ca^{2+}-sensing protein calmodulin and the calmodulin

kinase II (CaMKII) upstream of hVPS34 [63,64]. Secretion of the bacterial secreted PtdIns(3)P phosphatase SapM contributes to further decrease PtdIns(3)P levels on the *Mycobacterium*-containing compartment [65], completely inhibiting phagosomal maturation. An additional mycobacterial secreted enzyme, MptpB (a *Listeria* LipA homologue), has been shown *in vitro* to exhibit phosphatase activity towards phosphotyrosine, phosphoserine/threonine as well as PtdIns(3)P, PtdIns(4)P, PtdIns(5)P and PtdIns(3,5)P_2 [29], and it is required for *Mycobacterium* survival in macrophages [66] but its potential contribution to phagosomal remodeling remains to be established. How *Mycobacterium* effectors reach their targets on the cytosolic side of vacuoles has not been fully documented: the use of the membrane-impermeant molybdate inhibits the activity of SapM on PtdIns(3)P, suggesting that membrane translocation is an important determinant for SapM function [65], but the potential mechanism for cytosolic export is unknown for SapM and also MptpB; ManLAM, as PIM, integrates into phagosomal membranes [55] but as mentioned above, its actual target and precise mechanism of action has not been elucidated. Of note, *Mycobacterium* secretes actively vesicles which contain many immunologically active molecules [67] and which act as potential vehicles to deliver effectors to diverse cellular locations including the cytosolic side of vesicles.

While *Mycobacterium* subverts phosphoinositide metabolism to promote its intracellular survival and replication, specific phosphoinositide species are also involved in the regulation of cellular mechanisms that limit mycobacterial infection. Redirection of the bacterial-containing compartment to autophagy was shown to overcome the mycobacterial-induced block of phagosome maturation, counteracting intracellular survival of the pathogen [68] and the GTPase Irgm1 was implicated in the generation of large LC3-II-positive autophagosomes in which *Mycobacterium* is degraded [69]. Irgm1 targeting to bacterial containing-phagosomes has been shown to be dependent on the class IA PI3-kinase, the phosphoinositide phosphatase SHIP1 and their respective phosphoinositide products PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 [70]. The host cell-derived PtdIns(3,4,5)P_3 phosphatase PTEN has recently been shown to limit intracellular mycobacterial replication in diverse human cell types by inhibiting PI3K/AKT signaling to mTOR through an autophagy-independent mechanism [71].

Salmonella enterica

The genus *Salmonella* comprises two species, *S. bongori* and *S. enterica*, the latter comprising more than 2000 serovars including *S. enterica* Typhi and *S. enterica* Paratyphi, responsible of the systemic typhoid and paratyphoid fevers [72] as well as *S. enterica* Typhimurium, the agent of typhoid fever in mice [73]. *Salmonella* invades epithelial cells and survives within macrophages in modified late endosomal compartments [74]. While translocation to the host cell cytoplasm has been reported for *Salmonella* [75,76], interactions with phosphoinositides have been described for the early invasion stages and for the maturation of the *Salmonella*-containing vacuole.

The *Salmonella* pathogenicity islands (SPI) 1 and 2 encode two independent T3SSs which control cell invasion and maturation of the *Salmonella* vacuole [77-79]. The SPI-1 effector SigD/SopB is a homologue of IpgD from *Shigella*, for which divergent biological activities have been proposed to date, both at early and late stages of the infection process. Hydrolysis of Ins(1,3,4,5,6)P_5 and production of Ins(1,4,5,6)P_4 via SigD/SopB-dependent and -independent mechanisms leads to host cytoskeletal rearrangements required for *Salmonella* entry within host cells in a Cdc42-dependent manner [80]; however, the host signaling targets regulated by Ins(1,4,5,6)P_4 upstream of Cdc42 have not been identified yet. During bacterial internalization, detachment of the newly formed *Salmonella* vacuole from the plasma membrane requires the hydrolysis of PtdIns(4,5)P_2 by SigD/SopB, reducing the membrane tension through removal of actin and associated proteins [81]; this PtdIns(4,5)P_2 dephosphorylation plays an additional role during phagosomal maturation as it reduces the vacuolar membrane surface charge, inhibiting the recruitment of several members of the Rab family and preventing fusion events with degradative lysosomal compartments [82]. Maturation of the *Salmonella* vacuole is characterized by the on/off cyclic accumulation/dissociation of PtdIns(3)P from its surface [83,84], produced in part by the class III PI 3-kinase Vps34 in a SigD/SopB and Rab5-dependent manner [85,86]. The PtdIns(3)P effector sorting nexin 1 governs the tubular-based remodeling of the *Salmonella*-containing compartment, which is critical for bacterial intracellular proliferation [87]. Sustained survival of *Salmonella* infected cells is achieved through the SigD/SopB-dependent hydrolysis of PtdIns(3,4,5)P_3 and production of PtdIns(3,4)P_2 which activates Akt [88,89].

Enteritis induced by non-typhoid *Salmonella* is characterized by fluid secretion and inflammatory responses in the infected ileum; in the case of *Salmonella dublin* infection, it has been proposed that SigD/SopB promotes fluid secretion by invaded intestinal cells by hydrolyzing Ins(1,3,4,5,6)P_5 to yield Ins(1,4,5,6)P_4, a molecule which increases chloride secretion, as well as by hydrolyzing PtdIns(3,4,5)P_3, which inhibits chloride secretion [90,91].

Legionella pneumophila

Legionella pneumophila was discovered in 1977 as the agent of a pneumonia known as the ‘legionnaires disease‘ [92,93]. In the environment, *Legionella* is normally associated to soil amoeba [94]. Upon inhalation in humans it multiplies intracellularly within blood monocytes and alveolar macrophages [95], causing respiratory problems. The *dot/icm* genes encode a type IV secretion system (T4SS) that translocates bacterial effectors into host phagocytes and controls the biogenesis of the *Legionella*-containing compartment [96,97] and its stability, as mutants of this system are found in the cytoplasm of host cells [98,99].

Legionella manipulates phosphatidylinositol metabolism to promote not only evasion from the endocytic pathway, but also to foster interactions with the endoplasmic reticulum and secretory pathways [100]. For example, the T4SS effector RidL localizes to the bacterial vacuole and binds the Vps29 retromer as well as PtdIns(3)P to block trafficking from endosomes towards the Golgi [101]. The T4SS secreted phosphatase SidP, which hydrolyzes PtdIns(3)P and PtdIns(3,5)P_2 *in*

vitro [102], may contribute to bacterial evasion of the endocytic/phagocytic pathway by depletion of PtdIns(3)P levels on the bacterial-containing compartment, inhibiting the recruitment of molecules such as EEA1, Hrs of SNX1 which would foster endosomal fusion [103]. Accumulation of PtdIns(4)P on the *Legionella* vacuole [103] on the other hand, may be achieved by the T4SS effector SidF, which displays 3-phosphatase activity towards PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 *in vitro* [104], and by the activity of a host cell class IIIβ PI 4-kinase [105]. This PI4P pool recruits SdcA and SidC, which promotes the recruitment of endoplasmic reticulum vesicles to *Legionella* vacuoles [106,107] as well as SidM/DrrA, which presents both guanosine exchange factor (GEF) and guanosine dissociation inhibitor (GDI) activities for the small GTPase Rab1 [108-110] and would also contribute to the tethering of vesicles during the endoplasmic reticulum-to-Golgi transport. LidA, another T4SS effector, may bind PtdIns(4)P but interacts preferentially with PI3P and also ampylated Rab1 [105,111] and favors additional interactions of the *Legionella* vacuole with the endoplasmic reticulum.

The host cell PI 5-phosphatase OCRL binds to the *Legionella* vacuole in association with the T4SS effector/PI3P-ligand LpnE, and it restricts bacterial intracellular growth [112] via a still non-identified mechanism.

Yersinia

The genus *Yersinia* harbors 17 species of Gram-negative bacteria, among which two food-borne pathogens, *Y. enterocolitica* and *Y. pseudotuberculosis,* responsible for gastrointestinal infections [113], and an arthropod-borne pathogen *Y. pestis*, the etiologic agent of the plague [114]. *Yersinia* spp. are able to induce their internalization into non-professional phagocytes, but during infection the bacterium is essentially extracellular as it is able to block macrophage phagocytosis through injection of T3SS effectors [115].

Interaction between the *Yersinia* surface protein invasin and β1 chains from integrins allows bacterial internalization in epithelial cells [116,117]. Downstream of β1-integrins, class IA PI 3-kinase activity is required for entry (Schulte et al 1998) and actin rearrangements are promoted by Rac1 via the Arp2/3 complex in a N-WASP-independent manner [118]. It has been initially proposed that Rac1, together with Arf6, favors the surface recruitment of a PIP5Kα and the focal production of PtdIns(4,5)P_2 at bacterial entry sites, which will act as a scaffold for actin-remodeling proteins [119]. More recently, Sarantis *et al.* have shown that the PtdIns(4,5)P_2-rich compartment surrounding invading *Yersinia* is not fully separated from the plasma membrane, and the activity of class IA PI 3-kinase is necessary to promote the recruitment of Rab5 and of the host 5-phosphatases OCRL and Inpp5b, which cleave the PtdIns(4,5)P_2 and allow vacuolar fission from the plasma membrane [120].

CONCLUSIONS

As demonstrated in this overview, bacterial intracellular pathogens exploit host cell phosphoinositides in many various ways. While the study of pathogen interactions with host signaling cascades, and in particular phosphoinositide signaling pathways, allows to understand the molecular basis of infection, bacterial intracellular pathogens can also be considered as molecular tools to discover novel cellular processes: this is the case, for example, of the Ca^{2+}/calmodulin/calmodulin kinase II cascade involved in control of the type III PI 3-kinase hVPS34, which was discovered by studying the adaptation of *Mycobacterium* to the intracellular environment [121]. Novel signaling functions for PtdIns(5)P in the inhibition of receptor degradation have been also uncovered by investigating the early events triggered by *Shigella* during host cell entry [46]. Novel tools for cell biology are also generated through these studies: the secreted *Legionella* T4SS effector SidM displays an unconventional PtdIns(4)P-binding motif that could be used to follow PtdIns(4)P dynamics in living cells [105]. It is probable that novel cellular functions for phosphoinositides will be discovered through the study of host-pathogen interactions.

ACKNOWLEDGMENTS

We would like to thank Matteo Bonazzi for technical assistance in correlative light electron microscopy. This work received financial support from the Institut Pasteur, Institut National de la Santé et de la Recherche Médicale (Unité 604), Institut National de la Recherche Agronomique (Unité Sous Contrat 2020), Fondation Louis Jeantet, European Research Council Advanced (Grant 233348 MODELIST), ERANET Pathogenomics Grant LISTRESS and Agence Nationale pour la Recherche. P.C. is a Howard Hughes Medical Institute Senior International Research Scholar. A.K. received a scholarship from the Pasteur-Paris University International Doctoral Program/Institut Carnot Maladies Infectieuses.

REFERENCES

[1] A.S. Fauci, D.M. Morens, The perpetual challenge of infectious diseases, N Engl J Med. 366 (2012) 454–461. doi:10.1056/NEJMra1108296.

[2] J. Pizarro-Cerdá, P. Cossart, Bacterial Adhesion and Entry into Host Cells, Cell. 124 (2006) 715–727. doi:10.1016/j.cell.2006.02.012.

[3] J. Pizarro-Cerdá, P. Cossart, Subversion of phosphoinositide metabolism by intracellular bacterial pathogens, Nat Cell Biol. 6 (2004) 1026–1033. doi:10.1038/ncb1104-1026.

[4] S.S. Weber, C. Ragaz, H. Hilbi, Pathogen trafficking pathways and host phosphoinositide metabolism, Mol Microbiol. 71 (2009) 1341–1352. doi:10.1111/j.1365-2958.2009.06608.x.

[5] P. Cossart, Illuminating the landscape of host-pathogen interactions with the bacterium Listeria monocytogenes, Proceedings of the National Academy of Sciences. (2011). doi:10.1073/pnas.1112371108.

[6] J. Pizarro-Cerdá, A. Kühbacher, P. Cossart, Entry of Listeria monocytogenes in mammalian epithelial cells: an updated view, Cold Spring Harb Perspect Med. 2 (2012). doi:10.1101/cshperspect.a010009.

[7] J. Pizarro-Cerdá, P. Cossart, Listeria monocytogenes membrane trafficking and lifestyle: the exception or the rule? Annu Rev Cell Dev Biol. 25 (2009) 649–670. doi:10.1146/annurev.cellbio.042308.113331.

[8] J. Mengaud, H. Ohayon, P. Gounon, Mege R-M, P. Cossart, E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells, Cell. 84 (1996) 923–932.

[9] Y. Shen, M. Naujokas, M. Park, K. Ireton, InIB-dependent internalization of Listeria is mediated by the Met receptor tyrosine kinase, Cell. 103 (2000) 501–510.

[10] K. Ireton, B. Payrastre, H. Chap, W. Ogawa, H. Sakaue, M. Kasuga, et al., A role for phosphoinositide 3-kinase in bacterial invasion, Science. 274 (1996) 780–782.

[11] K. Ireton, B. Payrastre, P. Cossart, The Listeria monocytogenes protein InlB is an agonist of mammalian phosphoinositide 3-kinase, J Biol Chem. 274 (1999) 17025–17032.

[12] H. Dokainish, B. Gavicherla, Y. Shen, K. Ireton, The carboxyl-terminal SH3 domain of the mammalian adaptor CrkII promotes internalization of Listeria monocytogenes through activation of host phosphoinositide 3-kinase, Cell Microbiol. 9 (2007) 2497–2516. doi:10.1111/j.1462-5822.2007.00976.x.

[13] H.L. Yin, P.A. Janmey, Phosphoinositide regulation of the actin cytoskeleton, Annu. Rev. Physiol. 65 (2003) 761–789. doi:10.1146/annurev.physiol.65.092101.142517.

[14] S. Seveau, T.N. Tham, B. Payrastre, A.D. Hoppe, J.A. Swanson, P. Cossart, A FRET analysis to unravel the role of cholesterol in Rac1 and PI 3-kinase activation in the InlB/Met signalling pathway, Cell Microbiol. 9 (2007) 790–803. doi:10.1111/j.1462-5822.2006.00832.x.

[15] H. Bierne, E. Gouin, P. Roux, P. Caroni, H.L. Yin, P. Cossart, A role for cofilin and LIM kinase in Listeria-induced phagocytosis, J Cell Biol. 155 (2001) 101–112. doi:10.1083/jcb.200104037.

[16] H. Bierne, H. Miki, M. Innocenti, G. Scita, F.B. Gertler, T. Takenawa, et al., WASP-related proteins, Abi1 and Ena/VASP are required for Listeria invasion induced by the Met receptor, J Cell Sci. 118 (2005) 1537–1547. doi:10.1242/jcs.02285.

[17] S. Jiwani, Y. Wang, G.C. Dowd, A. Gianfelice, P. Pichestapong, B. Gavicherla, et al., Identification of Components of the Host Type IA Phosphoinositide 3-Kinase Pathway That Promote Internalization of Listeria monocytogenes, Infect Immun. 80 (2012) 1252–1266. doi:10.1128/IAI.06082-11.

[18] A. Balla, T. Balla, Phosphatidylinositol 4-kinases: old enzymes with emerging functions, Trends Cell Biol. 16 (2006) 351–361. doi:10.1016/j.tcb.2006.05.003.

[19] J. Pizarro-Cerdá, B. Payrastre, Y.-J. Wang, E. Veiga, H.L. Yin, P. Cossart, Type II phosphatidylinositol 4-kinases promote Listeria monocytogenes entry into target cells, Cell Microbiol. 9 (2007) 2381–2390. doi:10.1111/j.1462-5822.2007.00967.x.

[20] T.N. Tham, E. Gouin, E. Rubinstein, C. Boucheix, P. Cossart, J. Pizarro-Cerdá, Tetraspanin CD81 is required for Listeria monocytogenes invasion, Infect Immun. 78 (2010) 204–209. doi:10.1128/IAI.00661-09.

[21] G.R.V. Hammond, M.J. Fischer, K.E. Anderson, J. Holdich, A. Koteci, T. Balla, et al., PI4P and PI(4,5)P2 Are Essential But Independent Lipid Determinants of Membrane Identity, Science. 337 (2012) 727–730. doi:10.1126/science.1222483.

[22] G.R.V. Hammond, M.P. Machner, T. Balla, A novel probe for phosphatidylinositol 4-phosphate reveals multiple pools beyond the Golgi, J Cell Biol. 183 (2014) 1061. doi:10.1083/jcb.201312072.dv.

[23] M. Lowe, Structure and function of the Lowe syndrome protein OCRL1, Traffic. 6 (2005) 711–719. doi:10.1111/j.1600-0854.2005.00311.x.

[24] K.S. Erdmann, Y. Mao, H.J. McCrea, R. Zoncu, S. Lee, S. Paradise, et al., A role of the Lowe syndrome protein OCRL in early steps of the endocytic pathway, Dev Cell. 13 (2007) 377–390. doi:10.1016/j.devcel.2007.08.004.

[25] D. Dambournet, M. Machicoane, L. Chesneau, M. Sachse, M. Rocancourt, A. El Marjou, et al., Rab35 GTPase and OCRL phosphatase remodel lipids and F-actin for successful cytokinesis, Nat Cell Biol. 13 (2011) 981–988. doi:10.1038/ncb2279.

[26] K. Ben El Kadhi, C. Roubinet, S. Solinet, G. Emery, S. Carréno, The Inositol 5-Phosphatase dOCRL Controls PI(4,5)P2 Homeostasis and Is Necessary for Cytokinesis, Curr Biol. 21 (2011) 1074–1079. doi:10.1016/j.cub.2011.05.030.

[27] A. Kühbacher, D. Dambournet, A. Echard, P. Cossart, J. Pizarro-Cerdá, Phosphatidylinositol 5-phosphatase oculocerebrorenal syndrome of Lowe protein (OCRL) controls actin dynamics during early steps of Listeria monocytogenes infection, Journal of Biological Chemistry. 287 (2012) 13128–13136. doi:10.1074/jbc.M111.315788.

[28] R. Kastner, O. Dussurget, C. Archambaud, E. Kernbauer, D. Soulat, P. Cossart, et al., LipA, a Tyrosine and Lipid Phosphatase Involved in the Virulence of Listeria monocytogenes, Infect Immun. 79 (2011) 2489–2498. doi:10.1128/IAI.05073-11.

[29] N. Beresford, S. Patel, J. Armstrong, B. Szöor, A.P. Fordham-Skelton, L. Tabernero, MptpB, a virulence factor from Mycobacterium tuberculosis, exhibits triple-specificity phosphatase activity, Biochem J. 406 (2007) 13. doi:10.1042/BJ20070670.

[30] J. Mengaud, C. Braun-Breton, P. Cossart, Identification of phosphatidylinositol-specific phospholipase C activity in Listeria monocytogenes: a novel type of virulence factor? Mol Microbiol. 5 (1991) 367–372.

[31] C. Geoffroy, J. Raveneau, J.L. Beretti, A. Lecroisey, J.A. Vázquez-Boland, J.E. Alouf, et al., Purification and characterization of an extracellular 29-kilodalton phospholipase C from Listeria monocytogenes, Infect Immun. 59 (1991) 2382–2388.

[32] I. Tattoli, M.T. Sorbara, C. Yang, S.A. Tooze, D.J. Philpott, S.E. Girardin, Listeria phospholipases subvert host autophagic defenses by stalling pre-autophagosomal structures, Embo J. 32 (2013) 3066–3078. doi:10.1038/emboj.2013.234.

[33] L.G. Tilney, D.A. Portnoy, Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, Listeria monocytogenes, J Cell Biol. 109 (1989) 1597–1608.

[34] C. Kocks, E. Gouin, M. Tabouret, P. Berche, H. Ohayon, P. Cossart, L. monocytogenes-induced actin assembly requires the actA gene product, a surface protein, Cell. 68 (1992) 521–531.

[35] G. Sidhu, W. Li, N. Laryngakis, E. Bishai, T. Balla, F. Southwick, Phosphoinositide 3-kinase is required for intracellular Listeria monocytogenes actin-based motility and filopod formation, J Biol Chem. 280 (2005) 11379–11386. doi:10.1074/jbc.M414533200.

[36] M.M. Levine, K.L. Kotloff, E.M. Barry, M.F. Pasetti, M.B. Sztein, Clinical trials of Shigella vaccines: two steps forward and one step back on a long, hard road, Nat Rev Micro. 5 (2007) 540–553. doi:10.1038/nrmicro1662.

[37] B. Marteyn, N.P. West, D.F. Browning, J.A. Cole, J.G. Shaw, F. Palm, et al., Modulation of Shigella virulence in response to available oxygen in vivo, Nature. 465 (2010) 355–358. doi:10.1038/nature08970.

[38] S. Romero, G. Grompone, N. Carayol, J. Mounier, S. Guadagnini, M.-C. Prévost, et al., ATP-Mediated Erk1/2 Activation Stimulates Bacterial Capture by Filopodia, which Precedes Shigella Invasion of Epithelial Cells, Cell Host Microbe. 9 (2011) 508–519. doi:10.1016/j.chom.2011.05.005.

[39] A. Blocker, P. Gounon, E. Larquet, K. Niebuhr, V. Cabiaux, C. Parsot, et al., The tripartite type III secreton of Shigella flexneri inserts IpaB and IpaC into host membranes, J Cell Biol. 147 (1999) 683–693.

[40] N. Carayol, G. Tran Van Nhieu, The Inside Story of Shigella Invasion of Intestinal Epithelial Cells, Cold Spring Harb Perspect Med. 3 (2013) a016717–a016717. doi:10.1101/cshperspect.a016717.

[41] K. Niebuhr, S. Giuriato, T. Pedron, D.J. Philpott, F. Gaits, J. Sable, et al., Conversion of PtdIns(4,5)P(2) into PtdIns(5)P by the S.flexneri effector IpgD reorganizes host cell morphology, Embo J. 21 (2002) 5069–5078.

[42] J. Mounier, M.R. Popoff, J. Enninga, M.C. Frame, P.J. Sansonetti, G.T. Van Nhieu, The IpaC Carboxyterminal Effector Domain Mediates Src-Dependent Actin Polymerization during Shigella Invasion of Epithelial Cells, PLoS Pathog. 5 (2009) e1000271. doi:10.1371/journal.ppat.1000271.t001.

[43] L. Bougnères, S.E. Girardin, S.A. Weed, A.V. Karginov, J.-C. Olivo-Marin, J.T. Parsons, et al., Cortactin and Crk cooperate to trigger actin polymerization during Shigella invasion of epithelial cells, J Cell Biol. 166 (2004) 225–235. doi:10.1083/jcb.200402073.

[44] C. Konradt, E. Frigimelica, K. Nothelfer, A. Puhar, W. Salgado-Pabon, V. di Bartolo, et al., The Shigella flexneri Type Three Secretion System Effector IpgD Inhibits T Cell Migrationby Manipulating Host Phosphoinositide Metabolism, Cell Host Microbe. 9 (2011) 263–272. doi:10.1016/j.chom.2011.03.010.

[45] C. Pendaries, H. Tronchère, L. Arbibe, J. Mounier, O. Gozani, L. Cantley, et al., PtdIns(5)P activates the host cell PI3-kinase/Akt pathway during Shigella flexneri infection, Embo J. 25 (2006) 1024–1034. doi:10.1038/sj.emboj.7601001.

[46] D. Ramel, F. Lagarrigue, V. Pons, J. Mounier, S. Dupuis-Coronas, G. Chicanne, et al., Shigella flexneri Infection Generates the Lipid PI5P to Alter Endocytosis and Prevent Termination of EGFR Signaling, Science Signaling. 4 (2011) ra61–ra61. doi:10.1126/scisignal.2001619.

[47] A. Puhar, H. Tronchère, B. Payrastre, G.T. Van Nhieu, P.J. Sansonetti, A Shigella Effector Dampens Inflammation by Regulating Epithelial Release of Danger Signal ATP through Production of the Lipid Mediator PtdIns5P, Immunity. 39 (2013) 1121–1131. doi:10.1016/j.immuni.2013.11.013.

[48] D.G. Russell, Mycobacterium tuberculosis and the intimate discourse of a chronic infection, Immunol. Rev. 240 (2011) 252–268. doi:10.1111/j.1600-065X.2010.00984.x.

[49] L.S. Schlesinger, C.G. Bellinger-Kawahara, N.R. Payne, M.A. Horwitz, Phagocytosis of Mycobacterium tuberculosis is mediated by human monocyte complement receptors and complement component C3, J Immunol. 144 (1990) 2771–2780.

[50] J. Gatfield, J. Pieters, Essential role for cholesterol in entry of mycobacteria into macrophages, Science. 288 (2000) 1647–1650.

[51] S. Sturgill-Koszycki, P.H. Schlesinger, P. Chakraborty, P.L. Haddix, H.L. Collins, A.K. Fok, et al., Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase, Science. 263 (1994) 678–681.

[52] L.E. Via, D. Deretic, R.J. Ulmer, N.S. Hibler, L.A. Huber, V. Deretic, Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7, J Biol Chem. 272 (1997) 13326–13331.

[53] N. van der Wel, D. Hava, D. Houben, D. Fluitsma, M. van Zon, J. Pierson, et al., M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells, Cell. 129 (2007) 1287–1298. doi:10.1016/j.cell.2007.05.059.

[54] R. Simeone, A. Bobard, J. Lippmann, W. Bitter, L. Majlessi, R. Brosch, et al., Phagosomal Rupture by Mycobacterium tuberculosis Results in Toxicity and Host Cell Death, PLoS Pathog. 8 (2012) e1002507. doi:10.1371/journal.ppat.1002507.s009.

[55] W.L. Beatty, E.R. Rhoades, H.J. Ullrich, D. Chatterjee, J.E. Heuser, D.G. Russell, Trafficking and release of mycobacterial lipids from infected macrophages, Traffic. 1 (2000) 235–247.

[56] I. Vergne, R.A. Fratti, P.J. Hill, J. Chua, J. Belisle, V. Deretic, Mycobacterium tuberculosis phagosome maturation arrest: mycobacterial phosphatidylinositol analog phosphatidylinositol mannoside stimulates early endosomal fusion, Mol Biol Cell. 15 (2004) 751–760. doi:10.1091/mbc.E03-05-0307.

[57] J. Chua, V. Deretic, Mycobacterium tuberculosis reprograms waves of phosphatidylinositol 3-phosphate on phagosomal organelles, J Biol Chem. 279 (2004) 36982–36992. doi:10.1074/jbc.M405082200.

[58] R.A. Fratti, J.M. Backer, J. Gruenberg, S. Corvera, V. Deretic, Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest, J Cell Biol. 154 (2001) 631–644. doi:10.1083/jcb.200106049.

[59] O.V. Vieira, R.E. Harrison, C.C. Scott, H. Stenmark, D. Alexander, J. Liu, et al., Acquisition of Hrs, an Essential Component of Phagosomal Maturation, Is Impaired by Mycobacteria, Mol Cell Biol. 24 (2004) 4593–4604. doi:10.1128/MCB.24.10.4593-4604.2004.

[60] M. Komada, P. Soriano, Hrs, a FYVE finger protein localized to early endosomes, is implicated in vesicular traffic and required for ventral folding morphogenesis, Genes Dev. 13 (1999) 1475–1485.

[61] A. Simonsen, R. Lippé, S. Christoforidis, J.M. Gaullier, A. Brech, J. Callaghan, et al., EEA1 links PI(3)K function to Rab5 regulation of endosome fusion, Nature. 394 (1998) 494–498. doi:10.1038/28879.

[62] F.T. Mu, J.M. Callaghan, O. Steele-Mortimer, H. Stenmark, R.G. Parton, P.L. Campbell, et al., EEA1, an early endosome-associated protein. EEA1 is a conserved alpha-helical peripheral membrane protein flanked by cysteine “fingers” and contains a calmodulin-binding IQ motif, J Biol Chem. 270 (1995) 13503–13511.

[63] R.A. Fratti, J. Chua, I. Vergne, V. Deretic, Mycobacterium tuberculosis glycosylated phosphatidylinositol causes phagosome maturation arrest, Proc Natl Acad Sci USA. 100 (2003) 5437–5442. doi:10.1073/pnas.0737613100.

[64] I. Vergne, J. Chua, V. Deretic, Tuberculosis Toxin Blocking Phagosome Maturation Inhibits a Novel Ca2+/Calmodulin-PI3K hVPS34 Cascade, Journal of Experimental Medicine. 198 (2003) 653–659. doi:10.1084/jem.20030527.

[65] I. Vergne, J. Chua, H.-H. Lee, M. Lucas, J. Belisle, V. Deretic, Mechanism of phagolysosome biogenesis block by viable Mycobacterium tuberculosis, Proc Natl Acad Sci USA. 102 (2005) 4033–4038. doi:10.1073/pnas.0409716102.

[66] N.J. Beresford, D. Mulhearn, B. Szczepankiewicz, G. Liu, M.E. Johnson, A. Fordham-Skelton, et al., Inhibition of MptpB phosphatase from Mycobacterium tuberculosis impairs

mycobacterial survival in macrophages, Journal of Antimicrobial Chemotherapy. 63 (2009) 928–936. doi:10.1093/jac/dkp031.

[67] R. Prados-Rosales, A. Baena, L.R. Martinez, J. Luque-Garcia, R. Kalscheuer, U. Veeraraghavan, et al., Mycobacteria release active membrane vesicles that modulate immune responses in a TLR2-dependent manner in mice, J Clin Invest. 121 (2011) 1471–1483. doi:10.1172/JCI44261DS1.

[68] M.G. Gutierrez, S.S. Master, S.B. Singh, G.A. Taylor, M.I. Colombo, V. Deretic, Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages, Cell. 119 (2004) 753–766. doi:10.1016/j.cell.2004.11.038.

[69] S.B. Singh, A.S. Davis, G.A. Taylor, V. Deretic, Human IRGM induces autophagy to eliminate intracellular mycobacteria, Science. 313 (2006) 1438–1441. doi:10.1126/science.1129577.

[70] S. Tiwari, H.-P. Choi, T. Matsuzawa, M. Pypaert, J.D. MacMicking, Targeting of the GTPase Irgm1 to the phagosomal membrane via PtdIns(3,4)P, Nature Publishing Group. 10 (2009) 907–917. doi:10.1038/ni.1759.

[71] G. Huang, G. Redelman-Sidi, N. Rosen, M.S. Glickman, X. Jiang, Inhibition of Mycobacterial Infection by the Tumor Suppressor PTEN, Journal of Biological Chemistry. 287 (2012) 23196–23202. doi:10.1074/jbc.M112.351940.

[72] J.A. Crump, E.D. Mintz, Global Trends in Typhoid and Paratyphoid Fever, Clin Infect Dis. 50 (2010) 241–246. doi:10.1086/649541.

[73] D. House, A. Bishop, C. Parry, G. Dougan, J. Wain, Typhoid fever: pathogenesis and disease, Curr. Opin. Infect. Dis. 14 (2001) 573–578.

[74] F. García-del Portillo, B.B. Finlay, Targeting of Salmonella typhimurium to vesicles containing lysosomal membrane glycoproteins bypasses compartments with mannose 6-phosphate receptors, J Cell Biol. 129 (1995) 81–97.

[75] C.R. Beuzón, S. Méresse, K.E. Unsworth, J. Ruíz-Albert, S. Garvis, S.R. Waterman, et al., Salmonella maintains the integrity of its intracellular vacuole through the action of SifA, Embo J. 19 (2000) 3235–3249. doi:10.1093/emboj/19.13.3235.

[76] P. Malik-Kale, S. Winfree, O. Steele-Mortimer, The Bimodal Lifestyle of Intracellular Salmonella in Epithelial Cells: Replication in the Cytosol Obscures Defects in Vacuolar Replication, PLoS ONE. 7 (2012) e38732. doi:10.1371/journal.pone.0038732.s008.

[77] J.E. Galán, R. Curtiss, Cloning and molecular characterization of genes whose products allow Salmonella typhimurium to penetrate tissue culture cells, Proc Natl Acad Sci USA. 86 (1989) 6383–6387.

[78] E.A. Groisman, H. Ochman, Cognate gene clusters govern invasion of host epithelial cells by Salmonella typhimurium and Shigella flexneri, Embo J. 12 (1993) 3779–3787.

[79] J.E. Shea, M. Hensel, C. Gleeson, D.W. Holden, Identification of a virulence locus encoding a second type III secretion system in Salmonella typhimurium, Proc Natl Acad Sci USA. 93 (1996) 2593–2597.

[80] D. Zhou, L.M. Chen, L. Hernandez, S.B. Shears, J.E. Galán, A Salmonella inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin

cytoskeleton rearrangements and bacterial internalization, Mol Microbiol. 39 (2001) 248–259.

[81] M.R. Terebiznik, O.V. Vieira, S.L. Marcus, A. Slade, C.M. Yip, W.S. Trimble, et al., Elimination of host cell PtdIns(4,5)P2 by bacterial SigD promotes membrane fission during invasion by Salmonella, Nat Cell Biol. 4 (2002) 766–773. doi:10.1038/ncb854.

[82] M.A. Bakowski, V. Braun, G.Y. Lam, T. Yeung, W. Do Heo, T. Meyer, et al., The Phosphoinositide Phosphatase SopB Manipulates Membrane Surface Charge and Trafficking of the Salmonella-Containing Vacuole, Cell Host Microbe. 7 (2010) 453–462. doi:10.1016/j.chom.2010.05.011.

[83] K. Pattni, M. Jepson, H. Stenmark, G. Banting, A PtdIns(3)P-specific probe cycles on and off host cell membranes during Salmonella invasion of mammalian cells, Curr Biol. 11 (2001) 1636–1642.

[84] C.C. Scott, P. Cuellar-Mata, T. Matsuo, H.W. Davidson, S. Grinstein, Role of 3-phosphoinositides in the maturation of Salmonella-containing vacuoles within host cells, J Biol Chem. 277 (2002) 12770–12776. doi:10.1074/jbc.M110399200.

[85] L.D. Hernandez, K. Hueffer, M.R. Wenk, J.E. Galán, Salmonella modulates vesicular traffic by altering phosphoinositide metabolism, Science. 304 (2004) 1805–1807. doi:10.1126/science.1098188.

[86] G.V. Mallo, M. Espina, A.C. Smith, M.R. Terebiznik, A. Aleman, B.B. Finlay, et al., SopB promotes phosphatidylinositol 3-phosphate formation on Salmonella vacuoles by recruiting Rab5 and Vps34, J Cell Biol. 182 (2008) 741–752. doi:10.1046/j.1365-2958.2001.02230.x.

[87] M.V. Bujny, P.A. Ewels, S. Humphrey, N. Attar, M.A. Jepson, P.J. Cullen, Sorting nexin-1 defines an early phase of Salmonella-containing vacuole-remodeling during Salmonella infection, J Cell Sci. 121 (2008) 2027–2036. doi:10.1242/jcs.018432.

[88] S.L. Marcus, M.R. Wenk, O. Steele-Mortimer, B.B. Finlay, A synaptojanin-homologous region of Salmonella typhimurium SigD is essential for inositol phosphatase activity and Akt activation, FEBS Lett. 494 (2001) 201–207.

[89] O. Steele-Mortimer, L.A. Knodler, S.L. Marcus, M.P. Scheid, B. Goh, C.G. Pfeifer, et al., Activation of Akt/protein kinase B in epithelial cells by the Salmonella typhimurium effector sigD, J Biol Chem. 275 (2000) 37718–37724. doi:10.1074/jbc.M008187200.

[90] F.A. Norris, M.P. Wilson, T.S. Wallis, E.E. Galyov, P.W. Majerus, SopB, a protein required for virulence of Salmonella dublin, is an inositol phosphate phosphatase, Proc Natl Acad Sci USA. 95 (1998) 14057–14059.

[91] E.E. Galyov, M.W. Wood, R. Rosqvist, P.B. Mullan, P.R. Watson, S. Hedges, et al., A secreted effector protein of Salmonella dublin is translocated into eukaryotic cells and mediates inflammation and fluid secretion in infected ileal mucosa, Mol Microbiol. 25 (1997) 903–912.

[92] J.E. McDade, C.C. Shepard, D.W. Fraser, T.R. Tsai, M.A. Redus, W.R. Dowdle, Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease, N Engl J Med. 297 (1977) 1197–1203. doi:10.1056/NEJM197712012972202.

[93] D.J. Brenner, A.G. Steigerwalt, J.E. McDade, Classification of the Legionnaires' disease bacterium: Legionella pneumophila, genus novum, species nova, of the family Legionellaceae, familia nova, Ann. Intern. Med. 90 (1979) 656–658.

[94] T.J. Rowbotham, Preliminary report on the pathogenicity of Legionella pneumophila for freshwater and soil amoebae, J. Clin. Pathol. 33 (1980) 1179–1183.

[95] M.A. Horwitz, Formation of a novel phagosome by the Legionnaires' disease bacterium (Legionella pneumophila) in human monocytes, J Exp Med. 158 (1983) 1319–1331.

[96] G. Segal, M. Purcell, H.A. Shuman, Host cell killing and bacterial conjugation require overlapping sets of genes within a 22-kb region of the Legionella pneumophila genome, Proc Natl Acad Sci USA. 95 (1998) 1669–1674.

[97] J.P. Vogel, Conjugative Transfer by the Virulence System of Legionella pneumophila, Science. 279 (1998) 873–876. doi:10.1126/science.279.5352.873.

[98] E.A. Creasey, R.R. Isberg, The protein SdhA maintains the integrity of the Legionella-containing vacuole, Proceedings of the National Academy of Sciences. 109 (2012) 3481–3486. doi:10.1073/pnas.1121286109.

[99] M. Molmeret, M. Santic, R. Asare, R.A. Carabeo, Y.A. Kwaik, Rapid Escape of the dot/icm Mutants of Legionella pneumophila into the Cytosol of Mammalian and Protozoan Cells, Infect Immun. 75 (2007) 3290–3304. doi:10.1128/IAI.00292-07.

[100] H. Hilbi, Anchors for effectors: subversion of phosphoinositide lipids by Legionella, (2011) 1–8. doi:10.3389/fmicb.2011.00091/abstract.

[101] I. Finsel, C. Ragaz, C. Hoffmann, C.F. Harrison, S. Weber, V.A. van Rahden, et al., The Legionella Effector RidL Inhibits Retrograde Traffickingto Promote Intracellular Replication, Cell Host Microbe. 14 (2013) 38–50. doi:10.1016/j.chom.2013.06.001.

[102] L. Toulabi, X. Wu, Y. Cheng, Y. Mao, Identification and Structural Characterization of a Legionella Phosphoinositide Phosphatase, Journal of Biological Chemistry. 288 (2013) 24518–24527. doi:10.1074/jbc.M113.474239.

[103] S. Weber, M. Wagner, H. Hilbi, Live-Cell Imaging of Phosphoinositide Dynamics and Membrane Architecture during Legionella Infection, mBio. 5 (2013) e00839–13–e00839–13. doi:10.1128/mBio.00839-13.

[104] F. Hsu, W. Zhu, L. Brennan, L. Tao, Z.-Q. Luo, Y. Mao, Structural basis for substrate recognition by a unique Legionella phosphoinositide phosphatase, Proceedings of the National Academy of Sciences. 109 (2012) 13567–13572. doi:10.1073/pnas.1207903109.

[105] E. Brombacher, S. Urwyler, C. Ragaz, S.S. Weber, K. Kami, M. Overduin, et al., Rab1 Guanine Nucleotide Exchange Factor SidM Is a Major Phosphatidylinositol 4-Phosphate-binding Effector Protein of Legionella pneumophila, Journal of Biological Chemistry. 284 (2009) 4846–4856. doi:10.1074/jbc.M807505200.

[106] S.S. Weber, C. Ragaz, K. Reus, Y. Nyfeler, H. Hilbi, Legionella pneumophila Exploits PI(4)P to Anchor Secreted Effector Proteins to the Replicative Vacuole, PLoS Pathog. 2 (2006) e46. doi:10.1371/journal.ppat.0020046.st002.

[107] C. Ragaz, H. Pietsch, S. Urwyler, A. Tiaden, S.S. Weber, H. Hilbi, The Legionella pneumophilaphosphatidylinositol-4 phosphate-binding type IV substrate SidC recruits

endoplasmic reticulum vesicles to a replication-permissive vacuole, Cell Microbiol. 10 (2008) 2416–2433. doi:10.1111/j.1462-5822.2008.01219.x.

[108] M.P. Machner, R.R. Isberg, A Bifunctional Bacterial Protein Links GDI Displacement to Rab1 Activation, Science. 318 (2007) 974–977. doi:10.1126/science.1149121.

[109] M.P. Machner, R.R. Isberg, Targeting of host Rab GTPase function by the intravacuolar pathogen Legionella pneumophila, Dev Cell. 11 (2006) 47–56. doi:10.1016/j.devcel.2006.05.013.

[110] T. Murata, A. Delprato, A. Ingmundson, D.K. Toomre, D.G. Lambright, C.R. Roy, The Legionella pneumophila effector protein DrrA is a Rab1 guanine nucleotide-exchange factor, Nat Cell Biol. 8 (2006) 971–977. doi:10.1038/ncb1463.

[111] M.R. Neunuebel, S. Mohammadi, M. Jarnik, M.P. Machner, Legionella pneumophila LidA affects nucleotide binding and activity of the host GTPase Rab1, J Bacteriol. 194 (2012) 1389–1400. doi:10.1128/JB.06306-11.

[112] S.S. Weber, C. Ragaz, H. Hilbi, The inositol polyphosphate 5-phosphatase OCRL1 restricts intracellular growth of Legionella, localizes to the replicative vacuole and binds to the bacterial effector LpnE, Cell Microbiol. 11 (2009) 442–460. doi:10.1111/j.1462-5822.2008.01266.x.

[113] P. Dube, Interaction of Yersinia with the Gut: Mechanisms of Pathogenesis and Immune Evasion, in: Current Topics in Microbiology and Immunology, Springer Berlin Heidelberg, Berlin, Heidelberg, 2009: pp. 61–91. doi:10.1007/978-3-642-01846-6_3.

[114] T. Butler, Plague into the 21st Century, Clin Infect Dis. 49 (2009) 736–742. doi:10.1086/604718.

[115] G.R. Cornelis, The Yersinia Ysc–Yop “Type III” weaponry, Nat Rev Mol Cell Biol. 3 (2002) 742–754. doi:10.1038/nrm932.

[116] R.R. Isberg, D.L. Voorhis, S. Falkow, Identification of invasin: a protein that allows enteric bacteria to penetrate cultured mammalian cells, Cell. 50 (1987) 769–778.

[117] R.R. Isberg, J.M. Leong, Multiple beta 1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells, Cell. 60 (1990) 861–871.

[118] M.A. Alrutz, A. Srivastava, K.W. Wong, C. D'Souza-Schorey, M. Tang, L.E. Ch'Ng, et al., Efficient uptake of Yersinia pseudotuberculosis via integrin receptors involves a Rac1-Arp 2/3 pathway that bypasses N-WASP function, Mol Microbiol. 42 (2001) 689–703.

[119] K.W. Wong, R.R. Isberg, Arf6 and Phosphoinositol-4-Phosphate-5-Kinase Activities Permit Bypass of the Rac1 Requirement for 1 Integrin-mediated Bacterial Uptake, Journal of Experimental Medicine. 198 (2003) 603–614. doi:10.1084/jem.20021363.

[120] H. Sarantis, D.M. Balkin, Pietro De Camilli, R.R. Isberg, J.H. Brumell, S. Grinstein, Yersinia Entry into Host Cells Requires Rab5-Dependent Dephosphorylation of PI(4,5)P2 and Membrane Scission, Cell Host Microbe. 11 (2012) 117–128. doi:10.1016/j.chom.2012.01.010.

[121] I. Vergne, J. Chua, S.B. Singh, V. Deretic, Cell Biology of *Mycobacterium tuberculosis* phagosome, Annu Rev Cell Dev Biol. 20 (2004) 367–394. doi:10.1146/annurev.cellbio.20.010403.114015.

[122] P. Cossart, A. Helenius, Endocytosis of Viruses and Bacteria, Cold Spring Harb. Perspect. Biol. 6 (2014) a016972. doi: 10.1101/cshperspect.a016972.

FIGURE LEGENDS

Figure 1. A. Internalization pathways of major intracellular bacterial pathogens in macrophages (*Mycobacterium*, *Legionella*), or in epithelial cells (*Salmonella, Shigella, Listeria, Yersinia*). Bacterial factors (colored) or cellular effectors (gray) related to phosphoinositides, required for intracellular invasion and replication, are depicted next to each bacterial internalization pathway (see main text for details). **B.** Phosphoinositide metabolism flowchart and host/bacterial enzymes (color-code according to **A**) modulating the production of different phosphoinositide species. *Listeria* PlcA cleaves phosphatidyl-inositol; *Listeria* also promotes the activity of class II PI4K to produce PtdIns(4)P and of class IA PI3K to produce PtdIns(3,4,5)P_3. *Mycobacterium* ManLAM blocks PI3K activity involved in the production of PI(3)P and SapM as well as MptpB cleave PtdIns(3)P on the bacterial phagosome; MptpB has also reported phosphatase activity for PtdIns(4)P, PtdIns(5)P and PtdIns(3,5)P_2 *in vitro*. According to different research groups, *Salmonella* SopB dephosphorylates PtdIns(3,5)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, while the SopB homolog in *Shigella*, IpgD, presents mainly PtdIns(4,5)P_2 phosphatase activity. The *Legionella* effector SidP dephosphorylates PtdIns(3)P and PtdIns(3,5)P_2, while SidF displays phosphatase activity towards PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. *Yersinia* requires the production of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 for efficient entry into host cells. Adapted from [122].

Figure 2. Colocalization between OCRL and clathrin-coated pits at *Listeria* entry sites. HeLa cells transfected with GFP-tagged OCRLA were infected with *Listeria* for 15 min and were fixed and processed first for fluorescence microscopy, and afterwards for transmission electron microscopy. Bacterial-containing compartments are decorated with OCRL-GFP (**a, b)** at locations that are also enriched for clathrin-coated pits (**e-g**).