Modification of phosphoinositides by the Shigella effector IpgD during host cell infection

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Shigella, the causative agent of bacillary dysentery, subvert cytoskeletal and trafficking processes to invade and replicate in epithelial cells using an arsenal of bacterial effectors translocated through a type III secretion system. Here, we review the various roles of the type III effector IpgD, initially characterized as phosphatidylinositol 4,5 bisphosphate (PI4,5P2) 4-phosphatase. By decreasing PI4,5P2 levels, IpgD triggers the disassembly of cortical actin filaments required for bacterial invasion and cell migration. PI5P produced by IpgD further stimulates signaling pathways regulating cell survival, macropinosome formation, endosomal trafficking and dampening of immune responses. Recently, IpgD was also found to exhibit phosphotransferase activity leading to PI3,4P2 synthesis adding a new flavor to this multipotent bacterial enzyme. The substrate of IpgD, PI4,5P2 is also the main substrate hydrolyzed by endogenous phospholipases C to produce inositoltriphosphate (InsP3), a major Ca2+ second messenger. Hence, beyond the repertoire of effects associated with the direct diversion of phoshoinositides, IpgD indirectly down-regulates InsP3-mediated Ca2+ release by limiting InsP3 production. Furthermore, IpgD controls the intracellular lifestyle of Shigella promoting Rab8/11-dependent recruitment of the exocyst at macropinosomes to remove damaged vacuolar membrane remnants and promote bacterial cytosolic escape. IpgD thus emerges as a key bacterial effector for the remodeling of host cell membranes.

KEYWORDS
Shigella, IpgD, phosphatidyl-inositol polyphosphate, phosphatase, invasion, dissemination, inflammation, phosphotransferase
Introduction

Invasive pathogens subvert cytoskeletal processes to promote their internalization by normally non-phagocytic cells. Once inside a targeted host cell, the pathogens may reside inside a membrane-bound bacterial-containing vacuole (BCV) or escape from this compartment. Diversion of endocytic processes has been mostly described for pathogens living inside BCVs. Recent evidence indicates that freely replicating bacteria within the cell cytosol, such as Shigella, the causative agent of bacillary dysentery, also divert host trafficking to promote vacuolar escape. Because of their central role in cytoskeletal reorganization and endosomal trafficking, phosphoinositides are targeted by virulence factors from a variety of invasive bacteria (Payrastre et al., 2012; Pizarro-Cerdà et al., 2015). Among these, type III secretion system (T3SS) effectors that are injected into the host cell cytosol are of particular interest. They alter general cellular signaling pathways, as well as exhibiting localized activities at bacterial entry sites (Jennings et al., 2017; Muthuramalingam et al., 2021).

The Shigella IpgD type III effector has been characterized in detail as a PI(3,4,5)P_3 4-phosphatase involved in key processes during host cell infection (Payrastre et al., 2012). IpgD-mediated effects have been linked to its PI_3P hydrolytic activity, decreased inositol (1, 4, 5) triphosphate (InsP_3) levels and increased production of PI5P (Payrastre et al., 2012). In a recent study, IpgD has been shown-similar to the Salmonella T3SS ortholog SopB to act as a phosphotransferase producing PI3,4P_2 questioning the precise enzymatic activity pertinent to host cell process subversion (Walpole et al., 2022). IpgD modulates local signals occurring at bacterial-host cell contact sites during invasion and during rupture of the phagocytic vacuole by regulating the activity of small GTPases and specific phosphoinositide levels associated with the BCV (López-Montero and Enninga, 2018; Morikoa et al., 2018). It also regulates Ca^{2+} signaling by depleting the second messenger InsP_3 from T3SS-targeted cells (Sun et al., 2017). Furthermore, IpgD is the main inducer of macropinosomes surrounding invading Shigella (Weiner et al., 2016; Chang et al., 2020). The kinetics of (i) invasion and (ii) vacuolar rupture are tightly linked, suggesting an interdependence between both processes (Enninga et al., 2005). Due to the overall complexity of the process, it remains poorly understood how IpgD mechanistically contributes to vacuolar rupture to promote Shigella intracellular replication. Additional T3SS effectors, such as IpgB1/2, IpaJ, VirB and IcsB, also modulate the dynamics of host cell membranes during bacterial entry (Handa et al., 2007; Hachani et al., 2008; Heindl et al., 2010; Burnaevskiy et al., 2013; Kühn et al., 2020).

IpgD thus exhibits multiple effects on phosphoinositides as well as on Ca^{2+} signaling. Here, we review the diverse processes targeted by IpgD with a particular emphasis on findings implicating IpgD in the diversion of macropinosome formation and endosomal trafficking leading to rupture of the bacterial containing vacuole.

Global IpgD-mediated effects on cell signaling and inflammation

Through its enzymatic activities, IpgD regulates many aspects of signaling involved in survival pathways and inflammatory processes. IpgD leads to the production of PI5P at Shigella invasion sites inducing the tyrosine kinase-dependent activation of class IA PI3K and activation of the Akt serine/threonine kinase regulating cell survival and proliferation (Pendaries et al., 2006) (Figure 1). In light of the recently described IpgD phosphotransferase activity, it is possible that Akt activation also occurs directly via increased PI 3,4P_2 levels triggered by IpgD (Walpole et al., 2022). Contributing to cell survival, IpgD specifically affects the dynamics of membranes at the host cell surface including endosome and macropinosome formation. Through increased PI5P levels at the plasma membrane of infected cells and a yet to be described mechanism, IpgD stimulates endocytosis of the EGF receptor (EGFR) and the cell adhesion molecule ICAM-1 (Boal et al., 2016) (Figure 1). While internalization is increased for these cell surface proteins, their outcome differs suggesting differential and specific cargo sorting for EGFR and ICAM-1 endosomes (Ramel et al., 2011; Boal et al., 2016). IpgD prevents the degradation of EGFR by blocking endosome maturation through the PI5P-dependent recruitment of the TOM1 adaptor protein at endosomal membranes (Boal et al., 2015). This results in the persistence of EGFR-early endosomes and persistent EGFR-signaling favoring the PI3K-Akt survival pathway (Boal et al., 2015) (Figure 1). The precise mechanism involved in the PI5P/TOM1-dependent blockade of endosome maturation remains to be characterized and appears unrelated to its described function in endosomal sorting. In contrast, as expected from canonical endosomal maturation, the increased internalization of ICAM-1 leads to its targeting to lysosomes and degradation (Figure 1). As a global result, IpgD stimulates the removal of ICAM-1 from the epithelial cell surface, thereby reducing neutrophil adhesion and clearance of infected cells (Boal et al., 2016). Through PI5P, IpgD also prevents the recruitment of immune cells and pro-inflammatory signals by limiting the release of extracellular ATP (eATP) from infected cells (Puhar et al., 2013) (Figure 1). Under basal conditions the eATP concentration is negligible, while being estimated in the order of 5–10 mM in the cytosol of host cells. Therefore, eATP is a well-characterized danger signal associated with cell injury, activation of pro-inflammatory cytokines and immune cell recruitment. Bacteria, such as Shigella, expressing a T3SS induce the release of eATP at the early stages of infection in the absence of detectable host cell lysis (Puhar et al., 2013). Shigella-induced eATP release results from an increase of cytosolic Ca^{2+} levels in infected cells leading to the opening of connexin hemichannels (Puhar et al., 2013). PI5P produced by IpgD prevents connexin hemichannel opening and the release of eATP associated with Shigella invasion, thereby dampening associated immune responses (Puhar et al., 2013).

Finally, Shigella negatively regulates the recruitment of neutrophils at infection sites through direct bacterial contact with
the immune cells (Konradt et al., 2011). While initially described during intestinal epithelial infection, it was also reported that injection of T3SS effectors occurred in neutrophils by transiently interacting bacteria in the absence of bacterial invasion (Konradt et al., 2011). Neutrophil migration requires dynamic reorganization of the actin cytoskeleton, as well as the membrane association of ERM family proteins acting as actin cytoskeletal linkers the hyaluronan receptor CD44 adhesion molecule. Critically, ERM protein association with the plasma membranes requires PIP2 (Konradt et al., 2011). Through PIP2 hydrolysis, IpgD prevents the membrane association of ERM proteins and neutrophil migration.

**IpgD as a key regulator of global and local Ca\(^{2+}\) signals**

Because PI4,5P\(_2\) is the main substrate utilized by PLCs to generate InsP\(_3\), IpgD also depresses InsP\(_3\) levels by decreasing PI4,5P\(_2\) levels. InsP\(_3\) is the key second messenger leading to Ca\(^{2+}\) release (Sun et al., 2017) (Figure 1). During prolonged infection kinetics, IpgD virtually abolishes cytosolic Ca\(^{2+}\) increase linked to release from internal stores. The inhibition of InsP\(_3\)-mediated signaling is expected to have versatile implications during infection. Notably, cytosolic Ca\(^{2+}\) increase is required for the activation of numerous Ca\(^{2+}\) proteases (Figure 1). As a result of its inhibitory effects on Ca\(^{2+}\) signaling, IpgD delays the Ca\(^{2+}\)-dependent activation of calpains and disassembly of cell adhesion structures, thereby preserving the integrity of *Shigella*-infected cells (Sun et al., 2017).

Beyond inhibition, IpgD also has modulatory effects on *Shigella*-induced Ca\(^{2+}\) signals at the early stages of bacterial invasion. Shortly upon contact with host cells, *Shigella* induces atypical Ca\(^{2+}\) microdomains required for actin polymerization at invasion sites (Tran Van Nhieu et al., 2013). These *Shigella*-induced Ca\(^{2+}\) microdomains correspond to local Ca\(^{2+}\) increase, which duration reaching up to several seconds depend on the dense actin meshwork triggered by bacteria at entry sites and localized aspects of PLC activation and InsP\(_3\) production at sites of bacteria-host cell membranes contact (Tran Van Nhieu et al., 2013). By limiting InsP\(_3\) production, IpgD favors the formation of Ca\(^{2+}\) microdomains at invasion sites, while limiting the elicitation of global Ca\(^{2+}\) responses during the early stages of *Shigella* invasion (Sun et al., 2017).

**Localized action of IpgD-stimulating actin polymerization during *Shigella* entry**

During contact between *Shigella* and target epithelial cells, ruffles are formed locally in the near surrounding of the bacteria. While bacterial invasion was initially described to occur through...
Another interaction partner of IpgD could be the BAR domain protein TOCA-1 that also interacts with other invasion (Miller et al., 2018). Nevertheless, the implication of this interaction requires more investigation. The type III effector IpgB1 is a RacGEF amplifying actin polymerization and ruffle formation at a distance from the bacterial contact-site. The translocon component IpaC triggers actin polymerization at the intimate bacterial contact site by triggering the recruitment of the Src tyrosine kinase, to form an actin coat structure reminiscent of phagocytic cups in macrophages (Valencia-Gallardo et al., 2015). The mechanistic links between Src and Rac activation remain to be characterized and could involve phospholipases C and Ca²⁺ signaling (Tran Van Nhieu et al., 2013).

The IpgD PIP₂ phosphatase activity favors actin polymerization at bacterial invasion sites. Seminal studies attributed this observation to the hydrolysis of PIP₂, leading to disassembly of cortical actin and stimulating de novo actin polymerization in close proximity to invading Shigella (Niebuhr et al., 2002). It became clear, however, that IpgD could regulate actin polymerization at invasion sites in various non-exclusive manners. Through PI3P production, IpgD may lead to the recruitment of the Rac GEF Tiam1 and Rac activation at membrane contacting bacteria (Viaud et al., 2014) (Figure 1). Binding of PI3P to Tiam-1 stimulates its intrinsic GEF activity and Rac activation in ruffles and endosomes (Viaud et al., 2014). Also, IpgD was shown to promote the recruitment of the Arf6 GEF ARNO, likely via an increase of PI(3,4,5)P₃ levels insuring a feed-back loop of Arf6 activation required for efficient invasion (Garza-Mayers et al., 2015) (Figure 1). While this was not investigated for Shigella, ARNO and Arf6 may potentiate actin polymerization in concert with the Rac-dependent recruitment of the Abi/WAVE complex as described for the Salmonella SopB ortholog (Humphreys et al., 2012; 2013). Another interaction partner of IpgD could be the BAR domain protein TOCA-1 that also interacts with other Shigella effectors; nevertheless, the implication of this interaction requires more investigation (Miller et al., 2018).

IpgD-mediated regulation of actin after host cell entry

Upon completion of the bacterial invasion process, a thick, so-called “cocoon” forms de novo around the BCV (Kühn et al., 2020) (Figure 1) This actin cocoon represents a unique structure implicating various cytoskeletal proteins and actin nucleation by the Arp2/3 complex. It is possible to speculate that through its Nε-fatty acetylase activity the T3SS effector IcsB is required for persistent activity of the Arp2/3 complex at the actin cocoon, while the initial recruitment of this actin nucleating complex depends on the activation of the Cdc42 small GTPase, at the onset of bacterial invasion (Liu et al., 2018). The role of the actin cocoon needs to be further investigated, however evidence shows that it is required for the efficient removal of damaged vacuolar remnants following rupture, that would otherwise impair bacterial spreading. Also, the cocoon may prevent recognition of the BCV by the cell autonomous immune system. Intriguingly, IpgD appears also involved in actin cocoon regulation, however it remains to be investigated how IpgD-mediated actin polymerization/dedepolymerization functions are spatio-temporally regulated.

Following vacuolar escape, Shigella replicates freely in the cell cytosol and disseminate from cell-to-cell. Cell-to-cell spreading is permitted by the bacterial ability to use actin-based motility, pushing bacteria-containing protrusion into adjacent cells (Weddle and Agaisse, 2018) (Figure 1). Upon lysis of the protrusion’s double membrane, Shigella re-iterates replication and cell-to-cell spreading thereby disseminating over large area of the epithelial layer. Key to this process, engulfment of the bacteria-containing protrusions requires the disassembly of actin filaments at the protrusion’s membranes. IpgD was shown to contribute to the down-regulation of actin structures in these protrusions, thus favoring their resolution into double-membrane containing secondary BCVs preceding bacterial escape into the cytosol within neighboring cells and bacteria dissemination (Figure 1). This highlights the diverse roles of IpgD during successive steps of Shigella invasion (Köseoğlu et al., 2022).

IpgD requirement during the events of vacuolar rupture

The BCV that englobes Shigella during its uptake differs from the macropinosytic vesicles in its close proximity, reinforcing differences between Shigella invasion and canonical macropinocytosis (Mellouk et al., 2014; Weiner et al., 2016; Chang et al., 2021). While bacterial invasion triggers the formation of ruffles and exocytic vesicles, the nascent BCV is formed in tight apposition with the bacterial body. The duality of macropinosome and BCV formation has been discovered through large volume correlative light and electron microscopy (CLEM) studies (Weiner et al., 2016; Weiner and Enninga, 2019). The newly formed macropinosomes cluster around the BCV within minutes upon bacterial invasion, however fusion between both compartments could not be observed. Furthermore, breaching of the BCV membrane takes place precisely at the time when it contacts macropinosomes. Macropinosomes-BCV contact sites can be visualized by 3D ultrastructural techniques, such as focused ion beam scanning EM. Studies on the formation of both compartments indicate that IpgD plays a main role in efficient vacuolar disruption (Mellouk et al., 2014; Chang et al., 2020).
mutants show similar internalization kinetics as WT Shigella, however, the usually observed massive damage of the BCV is delayed. The differences between simple BCV damage and the unwrapping of the damaged BCV leaflets can be measured combining two microscopic assays; fluorescently tagged galecins that get recruited to the inner leaflet of the BCV upon its damage show the morphology of BCV disassembly, while a sensitive enzymatic FRET reporter provides precise information on the initial damage of BCV damage. Two clear defects in relation to vacuolar rupture can be attributed to the $ipgD$ mutant Shigella strain: i, the absence of macropinosomes at bacterial entry sites; ii, the incapacity of invading Shigella to strip off BCV remnants that are typically transported several micrometers away upon BCV rupture. Potentially, the bacteria entrapped in broken BCVs are readily targeted by the xenophagy machinery. During these steps, forming macropinosomes become coated with Rab11, Rab8 and the exocyst complex (Chang et al., 2020), as shown by proteomics analysis of macropinosomes isolated from epithelial cells infected with Shigella (Chang et al., 2020; Stévenin et al., 2021) (Figure 1). The resulting Rab/exocyst coating is required for targeting these compartments to the BCV before its rupture. Upon Rab11 knockdown, depletion of key constituents of the exocyst complex, such as Exo70, or by using interfering peptides that hamper exocyst complex formation, the BCV and macropinosomes form less contacts. Also, damaged BCV membranes remain in close apposition to the bacteria (Mellouk et al., 2014; Chang et al., 2020), impairing intra- and inter-cellular bacterial spread as observed for a Shigella $ipgD$ mutant. Whether $IpgD$ acts only on the surrounding macropinosomes, on the BCV, or simultaneously on both compartments will require further investigation.

**$IpgD$ orthologs: The role of SopB during Salmonella invasion**

*Salmonella* SopB (or SigD) was already shown to be an ortholog of $IpgD$ more than two decades ago and it was further characterized as PI phosphatase (Norris et al., 1998). Both proteins exhibit an unstructured N-terminus likely involved in chaperone-binding and secretion by the T3SS, as well as a P-loop containing a Cys-X5-Arg motif that is a signature of the active site complex of Mg$^{2+}$-independent phosphatases (Norris et al., 1998; Niebuhr et al., 2002) (Figure 2A, red box). In addition, a discrete hydrophobic region involved in membrane interaction has been described for SopB and suggested for $IpgD$ (Patel et al., 2009; Figure 2A, yellow box MIM1). Using *in silico* computational methods for predicting topology of transmembrane 3D structures (DAS-TMpred and DeepEMPred), we confirmed MIM1 and identified a longer MIM2 for $IpgD$ (blue box) (Cserzö et al., 1997; Hsu and Mao, 2015; Weigele et al., 2017; Wang et al., 2022). We also predicted a similar MIM2 in SopB through its sequence and structural concordances (Figure 2A, blue box MIM2), that includes a motif conserved in host cell phosphatases formerly identified by Norris et al., 1998. (Figure 2A; brown empty box)). Using Pymol, we generated an *in silico* model of $IpgD$ that shows the spatial proximity of both MIMs to the P-loop (Figure 2B, left). This organization is similar to that of host phosphoinositides phosphatases, where MIMs are observed in close proximity to the active site and are presumed to facilitate accessibility to the phosphoinositide following membrane insertion (Hsu and Mao, 2015).

Further stressing similarities with host lipid phosphatases, the $IpgD$ surface surrounding the catalytic P-loop region shows a high density of positive charges likely involved in interaction with the negatively charged membranes embedding the phosphoinositide substrate (Figure 2B). Alphafold structure simulations of $IpgD$ and SopB demonstrate their high levels of homology with an identical organization of MIMs and conserved residues surrounding the P-loop region (Figure 2C, yellow, cyan and red). Together, this analysis also shows conserved structural features and domain organization between $IpgD$, SopB and phosphoinositide phosphatases (Hsu and Mao, 2015).

Functionally however, while *in vitro*, $IpgD$ preferentially targets for PI(4,5)P$_2$, SopB shows preferential activity towards PI(3,4)P$_2$. How the *in vitro* SopB phosphatase activity towards PI(3,4)P$_2$ relates to the increased PI(3,4)P$_2$ levels associated with the SopB phosphatase activity has remained unclear until recently, when SopB was identified as phosphotransferase similar to $IpgD$ (Walpole et al., 2022) (Figure 2). During host cell challenge with *Salmonella*, SopB has been found to increase PI3P levels, which sets it apart from the production of PI5P in the case of $IpgD$ (Hernandez et al., 2004). As for $IpgD$, SopB activates Akt but SopB-mediated Akt activation occurs through a process that requires PI(3,4)P$_2$ and is Wortmannin insensitive (Steele-Mortimer et al., 2000; Cooper et al., 2011). Local targeting of SopB at the onset of these events has been shown to depend on myosin 6 tethering to the entry site via SopE (Brooks et al., 2017). Akt activation promotes intracellular bacterial growth, modulates the immune response, enhances M cell growth within the intestinal epithelium, and potentially plays a role in infection associated carcinoma (Knodler et al., 2005; KUM et al., 2011; Tahoun et al., 2012; Scanu et al., 2015). More recently, SopB has also been linked with the intracellular niche formation of *Salmonella* during epithelial infection (Stévenin et al., 2021). SopB reprograms phosphoinositides at plasma membranes of *Salmonella* invasion sites (Hernandez et al., 2004; Piscitelli et al., 2016). Furthermore, SopB plays a major role in extracting membranes from the BCV during the early invasion phase through the formation of SNX3-positive tubules (Bujny et al., 2008; Braun et al., 2010).

Contrasting with Shigella invasion, *Salmonella*-induced macropinosomes can fuse with the BCV, a process that is SopB-independent. SopB appears rather involved in regulating the stability of the BCV, however through the regulation of membrane influx and eflux to and from the BCV (Stévenin et al., 2019). Therefore, despite the similarities of the enzymatic function of the Shigella and *Salmonella* effectors $IpgD$ and SopB, their mechanistic involvement in controlling the intracellular niche is distinct for both pathogens, perhaps due to differences in the composition of the macropinosomes forming around the respective BCVs.
Perspectives

The function of IpgD during *Shigella* has been studied for more than two decades and has revealed its crucial involvement during entry, intracellular niche formation, dissemination as well as host immune signaling. More generally, this reflects the importance of phosphoinositide reprogramming during the course of *Shigella* infection. Phosphoinositides are key

FIGURE 2
Conserved phosphoinositide phosphatase features of *Shigella* IpgD and *Salmonella* SopB. (A) Sequence alignments of MIMs and P-loop motives in IpgD and SopB. Bold characters: conserved residues. Yellow box: MIM1, blue box: MIM2, red box: P-loop. The MIMs were identified experimentally and through computational learning approaches (see text). (B) Left: Alphafold-based in silico 3D structure analysis of IpgD (Jumper et al., 2021) followed by PyMOL molecular visualization (Schrödinger and DeLano 2020) showing the P-loop and surrounding MIMs. Right: surface charge distribution. Blue: positive charges, red: negative charges. (C) High structural homologies between IpgD and SopB. Green: IpgD, purple: SopB, yellow: MIM1, cyan: MIM2, red: P-loop, black: conserved aminoacids. Left and Middle: Alphafold-based 3D structures of IpgD and SopB. Right: overlaid 3D structures showing their high homology. (D) Enzymatic reactions of IpgD and SopB. P(4,5)IP2 dephosphorylation either yields PI5P for both enzymes or PI3P in the case of SopB (left). The recently described phosphotransferase activity leads to PI(3,4)P2 production that could be further dephosphorylated in PI3P (right) (Walpole et al., 2022).
components linking membrane remodeling, cytoskeletal rearrangements, and complex signaling pathways including Ca²⁺. Based on recent discoveries reviewed here, more work is required to obtain a comprehensive understanding about the role of IpgD for Shigella infection. It will be particularly interesting to integrate the IpgD phosphotransferase activity with regards to its largely described phosphatase activity. As PI(3,4)P₂ and PI(5)P trigger different signals, it will be important to decipher what is the relevance for each of the different activities during cell infection by Shigella. A comprehensive analysis of the similarities and differences between the Shigella effector IpgD and SopB of Salmonella will also be useful to understand the differences of their respective intracellular lifestyles. In this regard, a detailed structure-function analysis targeting specific domains of these exciting effectors will be highly informative. Novel in silico approaches, such as AlphaFold are powerful tools to achieve this (Figure 2). Also, as IpgD acts locally at the entry site as well as globally within targeted cells, including at sites of cell-to-cell spread, it will be important to better understand the spatiotemporal organization of the action of IpgD. In this context, it would be intriguing to investigate whether IpgD gets post-translationally modified upon translocation into host cells as has been suggested for SopB (Patel et al., 2009; Popa et al., 2016). Finally, model tissues through organ-on-a-chip devices or cocultures (Grassart et al., 2019; Mitchell et al., 2020; Rey et al., 2020) may provide with important insights to confirm the role of IpgD in Shigella pathophysiology.

Author contributions

GTVN, JE, and PL-L contributed to conception and design of the study. GTVN, JE wrote the first draft of the manuscript. PL-L organized 3D structures and analysis. GTVN, JE, PL-L wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

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References

Boal, F., Mansour, R., Gayral, M., Saldan, E., Chicanne, G., Xuereb, J.-M., et al. (2015). TOM1 is a PISP effector involved in the regulation of endosomal maturation. J. Cell Sci. 128, 815–827. doi: 10.1242/jcs.166314
Boal, F., Puhar, A., Xuereb, J.-M., Kunduzova, O., Sansonetti, P. J., Payrastre, B., et al. (2016). PISP triggers ICAM-1 degradation in shigella infected cells, thus dampening immune cell recruitment. Cell Rep. 14, 750–759. doi: 10.1016/j.celrep.2015.12.079
Braun, V., Wong, A., Landecic, M., Hong, W. J., Grinstein, S., and Brumell, J. H. (2010). Sorting nexin 3 (SNX3) is a component of a tubular endosomal network induced by salmonella and involved in maturation of the salmonella-containing vacuole. Cell Microbes. 12, 1352–1367. doi: 10.1111/j.1462-5822.2010.01476.x
Brooks, A. B. E., Humphreys, D., Singh, V., Davidson, A. C., Arden, S. D., Buss, F., et al. (2017). MYO6 is targeted by salmonella virulence effectors to trigger PI3-kinase signaling and pathogen invasion into host cells. Proc. Natl. Acad. Sci. U.S.A. 114, 3915–3920. doi: 10.1073/pnas.1616418114
Bujny, M. V., Ewel, P. A., Humphrey, S., Attar, N., Jepson, M. A., and Cullen, P. J. (2008). Sorting nexin-1 defines an early phase of salmonella-containing vacuole-remodeling during salmonella infection. J. Cell Sci. 121, 2027–2036. doi: 10.1242/jcs.018432
Burnaevskiy, N., Fox, T. G., Pflimry, D. A., Ertelt, J. M., Weigele, R. B., Selyunin, A. S., et al. (2013). Proteolytic elimination of n-myristoyl modifications by the shigella virulence factor IpgD. Nature 496, 106–109. doi: 10.1038/nature12004
Chang, Y.-Y., Enninga, J., and Stévenin, V. (2021). New methods to decrypt emerging macropinosome functions during the host-pathogen crosstalk. Cell Microbes. 23, e13342. doi: 10.1111/cm.13342
Chang, Y.-Y., Stévenin, V., Duchateau, M., Giai Gianetto, Q., Hourdel, V., Rodrigues, C. D., et al. (2020). Shigella hijacks the exocyst to cluster macropinosomes for efficient vacuolar escape. PloS Pathog. 16, e1008822. doi: 10.1371/journal.ppat.1008822
Cooper, K. G., Winfree, S., Malik-Kale, P., Jolly, C., Irland, R., Knodler, L. A., et al. (2011). Activation of akt by the bacterial inositol phosphatase, SopB, is Wortmannin insensitive. PloS One 6, e22260. doi: 10.1371/journal.pone.0022260
Cserzo, M., Wallin, E., Simon, J., von Heijne, G., and Elofsson, A. (1997). Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: The dense alignment surface method. Protein Eng. 10, 673–676. doi: 10.1093/protein/10.6.673
Duménil, G., Sansonetti, P., and Tran Van Nhieu, G. (2000). Src tyrosine kinase activity down-regulates rho-dependent responses during shigella entry into
epithelial cells and stress fibre formation. J. Cell Sci. 113 (Pt 1), 71–80. doi: 10.1242/71371

Enninga, J., Mounier, J., Sanonetti, P., and Tran Van Nhieu, G. (2005) Secretion of type III effectors into host cells in real time. Nat. Methods 2, 959–965. doi: 10.1038/nmeth0402

Garza-Mayers, A. C., Miller, K. A., Russo, B. C., Nagla, D. V., and Goldberg, M. B. (2018). Shigella lateralization of ARoS activation during bacterial entry via an IgG3-mediated positive feedback loop. mBio 6, e02584–14. doi: 10.1128/mBio.02584–14

Grassart, A., Malardé, V., Goba, S., Sartori-Rupp, A., Kerns, J., Kralis, K., et al. (2019). Bioengineered human organ-on-Chip reveals intestinal microenvironment and mechanical forces impacting shigella infection. Cell Host Microbe 15, 435–444.e4. doi: 10.1016/j.chom.2019.08.007

Hachani, A., Biskri, L., Rossi, G., Marty, A., Menard, R., Sanonetti, P., et al. (2008). IgG1p and IgG2, two homologous effectors secreted via the mxi-spa type III secretion apparatus, cooperate to mediate polarized cell invasion and inflammatory potential of shigella flexneri. Microbes Infect. 10, 260–268. doi: 10.1156/j.micinf.2007.11.011

Handa, Y., Suzuki, M., Ohya, K., Iwai, H., Ishijima, N., Koleske, A. J., et al. (2007). Shigella IgG1p promotes bacterial entry through the ELMO-Dock180 machinery. Nat. Cell Biol. 9, 121–128. doi: 10.1038/nrb21526

Heindl, J. E., Saran, J., Yi, C., Leser, C. F., and Goldberg, M. B. (2010). Requirement for formin-induced actin polymerization during spread of shigella flexneri. Infect. Immun. 78, 193–203. doi: 10.1128/IAI.00252-09

Hernandez, L. D., Hueffer, K., Wenk, M. R., and Galán, J. E. (2004). Salmonella modulates Rap1c and Rab11c to alter phosphoinositide metabolism. Science 304, 1805–1807. doi: 10.1126/science.1069988

Hsu, F., and Mao, Y. (2015). The structure of phosphoinositide phosphatases: Insights into substrate specificity and catalysis. Biochim. Biophys. Acta 1851, 698–710. doi: 10.1016/j.bbalip.2014.09.011

Humphreys, D., Davidson, H., Hume, P. J., and Koronakis, V. (2012). Salmonella virulence effector SopE protects epithelial cells from apoptosis by sustained activation of Akt2 in salmonella enterica serovar typhimurium-induced gastroenterocolitis. PloS Pathog. 8, 1009157. doi:10.1371/journal.ppat.1009157

Kühn, S., Bergqvist, J., Gil, M., Valenzuela, C., Barrio, L., Lebreton, S., et al. (2015). Highly accurate protein structure prediction with AlphaFold. Nature 596, 382–391. doi:10.1038/nature12113

Knodler, L. A., Finlay, B. B., and Steele-Mortimer, O. (2005). The salmonella virulence effector SopF1 and host GEF ARNO cooperate to activate and recruit activate WAVE to trigger bacterial invasion. Cell Host Microbe 11, 129–139. doi: 10.1016/j.chom.2012.01.006

Leung, C. K., Hume, P. J., and Koronakis, V. (2013). Arf6 coordinates actin assembly through the WAVE complex, a mechanism usurped by salmonella to invade host cells. Proc. Natl. Acad. Sci. U.S.A. 110, 18880–18885. doi: 10.1073/pnas.1311680110

Liu, W., Zhou, Y., Peng, T., Zhou, P., Ding, X., Li, Z., et al. (2018). NLRP12 is a protein required for virulence of salmonella dublin, is an inositol phosphate phosphatase. Proc. Natl. Acad. Sci. U.S.A. 115, 9457–9469. doi:10.1073/pnas.1718839115

Miller, K. A., Garza-Mayers, A. C., Hume, P. J., Makin, L. E., and Koronakis, V. (2013). Arf6 and Arf1 interact in Salmonella enterica serovar typhimurium-induced gastrointestinal injury. J. Biol. Chem. 288, 9058–9064. doi:10.1074/jbc.M112588200

Puhar, A., Tronchère, H., Payrastre, B., Nhieu, G. T. V., and Sansonetti, P. J. (2011). Protective role of Shigella flexneri invasiongenerated the lipid PI5P to alter calcium responses during shigella flexneri infection. PLoS Pathog. 6, 1001846. doi:10.1371/journal.ppat.1001846

Puhar, A., Tronchère, H., Payrastre, B., Nhieu, G. T. V., and Sansonetti, P. J. (2011). Protective role of Shigella flexneri invasion generated the lipid PI5P to alter calcium responses during shigella flexneri infection. PLoS Pathog. 6, 1001846. doi:10.1371/journal.ppat.1001846

Ramel, D., Lagarrigue, F., Pons, V., Mounier, J., Dupuis-Coronas, S., Chianne, G., et al. (2013). Shigella flexneri infection generates the lipid PIP3 to alter endocytosis and prevent termination of EGFR signaling. Sci. Signal. 6, ra16. doi: 10.1126/scisignal.2001619

Rey, C., Chang, Y.-Y., Lafort-Lambert, P., Varela, H., Prox, C., Legendre, R., et al. (2020). Transcytosis subversion by m cell-to-enterocyte spread promotes shigella flexneri and listeria monocytogenes intracellular bacterial dissemination. PLoS Pathog. 16, e1008446. doi:10.1371/journal.ppat.1008446

Schrödingler, L., and Delano, W. (2020) PyMOL Available at: http://www. pymol.org/pymol

Steel-Mortimer, O., Knodler, L. A., Marcus, S. L., Scheid, M. P., Goh, B., Pfeifer, G. G., et al. (2000). Activation of akt1/protein kinase b in epithelial cells by the salmonella typhimurium effector protein sigd. J. Biol. Chem. 275, 37718–37724. doi:10.1074/jbc.M001887200

Sivasubramanian, M., Thirumalai, K., and Arthanari, H. R. (2015). Purification of infection-associated macropinosomes by magnetic isolation for proteomic characterization. Nat. Protoc. 10, 5220–5249. doi:10.1038/nprot.2015.507

Sun, C. H., Wacquier, A., Aguilar, D. I., Carayol, N., Denis, K., Boucherie, S., et al. (2017). The Salmonella typhimurium effector IpgD recodes Cdc2 signals during invasion of epithelial cells. EMBO J. 36, 2567–2580. doi:10.15252/embj.201696272

Tahoun, A., Mahajan, S., Paxton, E., Malterer, G., Donaldson, D. S., Wang, D., et al. (2012). Salmonella transforms follicle-associated epithelial cells into m cells to promote intestinal invasion. Cell Host Microbe 12, 645–656. doi:10.1016/j.chom.2012.10.009

Tran Van Nhieu, G., Kai Liu, B., Zhang, J., Pierre, F., Frignet, S., Sanonetti, P., et al. (2013). Actin-based confinement of calcium responses during shigella infection. Nat. Commun. 4, 1567–1–10. doi: 10.1038/ncomms2561

Van Nhieu et al. 10.3389/fcimb.2022.1012533
Valencia-Gallardo, C. M., Carayol, N., and Tran Van Nhieu, G. (2015). Cytoskeletal mechanics during shigella invasion and dissemination in epithelial cells. *Cell Microbiol.* 17, 174–182. doi:10.1111/cmi.12400

Viaud, J., Lagarrigue, F., Ramel, D., Allart, S., Chicanne, G., Ceccato, L., et al. (2014). Phosphatidylinositol 5-phosphate regulates invasion through binding and activation of Tiam1. *Nat. Commun.* 5 (4080), 1–17. doi:10.1038/ncomms5080

Walpole, G. F., Wacheco, J., Chauhan, N., Clark, J., Anderson, K. E., Abbas, Y. M., et al. (2022). Kinase-independent synthesis of 3-phosphorylated phosphoinositides by a phosphotransferase. *Nat. Cell Biol.* 24, 708–722. doi:10.1038/s41556-022-00895-y

Wang, L., Zhong, H., Xue, Z., and Wang, Y. (2022). Improving the topology prediction of α-helical transmembrane proteins with deep transfer learning. *Comput. Struct. Biotechnol. J.* 20, 1993–2000. doi:10.1016/j.csbj.2022.04.024

Weddle, E., and Agaisse, H. (2018). Principles of intracellular bacterial pathogen spread from cell to cell. *PloS Pathog.* 14, e1007380. doi: 10.1371/journal.ppat.1007380

Weigele, B. A., Orchard, R. C., Jimenez, A., Cox, G. W., and Alto, N. M. (2017). A systematic exploration of the interactions between bacterial effector proteins and host cell membranes. *Nat. Commun.* 8, 532. doi:10.1038/s41467-017-00700-7

Weiner, A., and Enninga, J. (2019). The pathogen-host interface in three dimensions: Correlative FIB/SEM applications. *Trends Microbiol.* 27, 426–439. doi:10.1016/j.tim.2018.11.011

Weiner, A., Mellouk, N., Lopez-Montero, N., Chang, Y.-Y., Souque, C., Schmitt, C., et al. (2016). Macropinosomes are key players in early shigella invasion and vacuolar escape in epithelial cells. *PloS Pathog.* 12, e1005602. doi: 10.1371/journal.ppat.1005602