Manipulation of host membranes by bacterial effectors

Hyeilin Ham, Anju Sreelatha* and Kim Orth*

Abstract | Bacterial pathogens interact with host membranes to trigger a wide range of cellular processes during the course of infection. These processes include alterations to the dynamics between the plasma membrane and the actin cytoskeleton, and subversion of the membrane-associated pathways involved in vesicle trafficking. Such changes facilitate the entry and replication of the pathogen, and prevent its phagocytosis and degradation. In this Review, we describe the manipulation of host membranes by numerous bacterial effectors that target phosphoinositide metabolism, GTPase signalling and autophagy.

Type III secretion system
(T3SS). A multisubunit, needle-like apparatus that is found in various Gram-negative bacterial pathogens of plants and animals, and penetrates the host cell membrane to translocate effectors into the host cytoplasm during infection.

Type IV secretion system
(T4SS). A multisubunit transporter complex that is found in various Gram-negative bacterial pathogens and delivers substrate molecules, including effector proteins and DNA, into the host cell.

Bacterial effectors
Proteins that are secreted by bacterial pathogens and used as virulence factors during infection.

Pilus
A hair-like projection that attaches one bacterium to another.

Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA. Correspondence to K.O.
e-mail: kim.orth@utsouthwestern.edu
**These authors contributed equally to this work.*
doi:10.1038/nrmicro2602
Published online 18 July 2011

Bacterial pathogens have evolved diverse strategies of evading the host's immune system to achieve a successful infection. Many Gram-negative bacteria possess a type III secretion system (T3SS) or a type IV secretion system (T4SS) to translocate certain proteins, termed bacterial effectors, into host cells to modulate various signalling pathways. The T3SS uses a multisubunit, needle-like apparatus that penetrates the host cell membrane and delivers effectors directly into the host cytoplasm[1]. The T4SS assembles a transport complex that is similar to a pilus but transfers both DNA and protein substrates[2–3]. Bacterial effectors subvert numerous eukaryotic activities for the benefit of the pathogen. For intracellular bacteria, these activities include facilitating entry into the host cell and promoting survival and replication of the bacteria in the cytoplasm or intracellular compartments. For example, *Salmonella enterica* subsp. *enterica* serovar Typhimurium can manipulate the endocytic pathway to create a replicative niche and prevent fusion with the lysosome. For extracellular bacteria such as *Vibrio parahaemolyticus*, effectors are essential for evading phagocytosis by disrupting the actin cytoskeleton or by inducing cell death pathways in host immune cells. Elucidating the mechanisms of action of these virulence factors provides a powerful tool for understanding host cell signalling pathways.

As shown in FIG. 1, intracellular bacterial pathogens invade non-phagocytic host cells such as intestinal epithelial cells using two mechanisms: zipper and trigger[4]. Bacteria using the zipper mechanism, such as *Yersinia* spp. and *Listeria monocytogenes*, express surface proteins that bind receptors on the host cell membrane on contact, inducing signalling cascades that reorganize the actin cytoskeleton to internalize the bacterium. The trigger mechanism, which is best characterized in *Shigella flexneri* and *S.* Typhimurium, employs the T3SS to deliver proteins across the host plasma membrane; these proteins directly interact with the cellular components that regulate actin dynamics. After internalization, the bacterium can either form an intracellular vacuole to replicate (as is the case for *S.* Typhimurium, *Mycobacterium tuberculosis* and *Legionella pneumophila*), or escape to the cytosol (as is the case for *S. flexneri* and *L. monocytogenes*). The bacterium-containing vacuoles are derived from host membranes such as endosomal vesicles and endoplasmic reticulum (ER). Many bacterial effectors interact with host endocytic pathways to maintain the integrity of the vacuole and control its maturation. For example, SifA, a T3SS effector from *S.* Typhimurium, induces the formation of *Salmonella*-induced filaments (Sifs), which are membrane tubules that protrude from *Salmonella*-containing vacuoles (SCVs) and extend along microtubules[5–8]. Sifs have an important role in recruiting vesicle membranes to maintain the integrity of SCVs[5–8]. Bacteria that escape to the cytosol interact with the actin polymerization machinery to migrate to the plasma membrane, where they can disseminate to neighbouring cells. Contrary to intracellular pathogens, extracellular bacteria such as *V. parahaemolyticus* and enteropathogenic *Escherichia coli* (EPEC) adhere to host cells and secrete T3SS effectors that reorganize the actin cytoskeleton in order to manipulate the plasma membrane for effective infection.

On infection, bacterial pathogens interact with host membranes through different mechanisms. The interaction between the bacterium and the host plasma membrane (and its embedded receptors) results in the activation of multiple host signalling pathways that can alter actin cytoskeleton dynamics or vesicle trafficking.

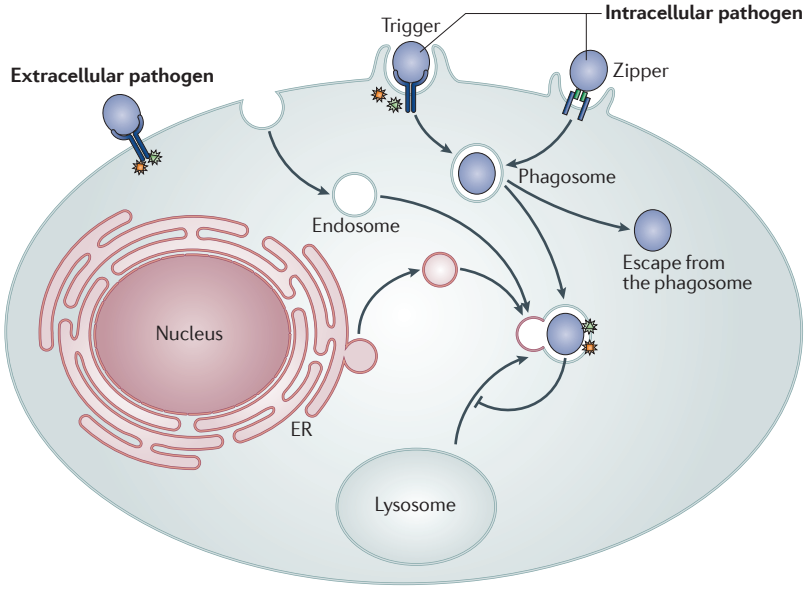

Figure 1 | **Lifestyles for pathogenic bacteria.** Intracellular bacterial pathogens invade non-phagocytic host cells through two mechanisms: zipper and trigger. The zipper mechanism uses bacterial surface proteins that bind receptors on the host cell membrane on contact, triggering a signalling cascade that reorganizes the actin cytoskeleton to internalize the bacterium. The trigger mechanism employs the bacterial type III secretion system (T3SS) or type IV secretion system (T4SS) to deliver proteins across the host plasma membrane to directly interact with the cellular components that regulate actin dynamics. After internalization, the bacterium can either persist in an intracellular vacuole that is derived from host cell or vesicle membranes, or escape to the cytosol. Extracellular pathogens secrete effectors that disrupt the host signalling system. ER, endoplasmic reticulum.

This Review focuses on three membrane-associated signalling events that are targeted by bacterial pathogens: phosphoinositide (PI) metabolism, GTPase signalling and autophagy. First, we explore the bacterial effectors that modulate PI metabolism to alter membrane dynamics and promote the maturation of bacterium-containing intracellular compartments. Second, we describe how effectors that target GTPase signalling can manipulate the actin cytoskeleton and endosomal trafficking. Last, we review bacterial strategies of subverting the autophagic pathway to their advantage.

PI metabolism and membrane dynamics

PIs are small lipids derived from phosphatidylinositol. As key components of cell membranes, PIs have essential roles in a wide range of cellular processes, such as membrane dynamics, actin cytoskeleton arrangements and vesicle trafficking (for reviews, see REFS 9–11). The differential distribution of PIs in cell membranes is tightly regulated by localized PI kinases and phosphatases, which interconvert diverse PI species. This dynamic diversity enables effective temporal and spatial regulation of membrane-associated signalling events. Owing to its involvement in a wide range of cellular functions, PI metabolism is often targeted by bacterial virulence factors that act as PI phosphatases or PI adaptor proteins (TABLE 1) (for reviews, see REFS 12–14). Pathogens target PI signalling for different purposes, such as internalization into host cells for replication in the cytoplasm (*S. flexneri*), disruption of plasma membrane integrity (*V. parahaemolyticus*), adhesion to the cell surface (EPEC) and internalization into specific intracellular compartments (*M. tuberculosis*, *L. pneumophila* and *S.* Typhimurium). In this section, we describe the bacterial effectors that manipulate plasma membrane dynamics and vesicle trafficking by subverting PI metabolism (FIG. 2).

Alteration of actin dynamics at host plasma membranes. PIs are key players in maintaining cell membrane structure by regulating the actin cytoskeleton underneath the plasma membrane and by tagging and targeting vesicles around the cell (BOX 1). The disruption of PI homeostasis at the plasma membrane by bacterial effectors can destabilize actin dynamics and alter the morphology of the membrane. This facilitates the entry of intracellular pathogens or enables extracellular pathogens to damage the cell by disrupting membrane integrity, eventually leading to rapid cell lysis in the later stage of infection.

The inositol phosphate phosphatase IpgD is an effector from the facultative intracellular pathogen *S. flexneri* that is directly translocated into host cells through a T3SS on contact with the cell surface[15]. IpgD hydrolyses phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) to produce PtdIns5P, preferentially early in the infection[16]. Owing to its regulatory role in the adhesion of the actin cytoskeleton to the cell cortex[17], removal of PtdIns(4,5)P_2 by IpgD decreases the tethering force of the plasma membrane to PtdIns(4,5)P_2-binding cytoskeletal-anchoring proteins, causing the extension of membrane filopodia and massive cell blebbing (observed as bubble-like protrusions)[18–20]. This reorganization of the actin cytoskeleton at the bacterial entry site promotes host cell uptake of the invasive pathogen.

A similar molecular mechanism is employed by the recently characterized T3SS effector VPA0450, from the extracellular pathogen *V. parahaemolyticus*[21]. VPA0450 contains catalytic motifs from inositol polyphosphate 5-phosphatases, which allow it to actively hydrolyse PtdIns(4,5)P_2 at the membrane surface. In contrast to IpgD, which produces PtdIns5P from PtdIns(4,5)P_2, VPA0450 hydrolyses the D5 phosphate, resulting in the production of PtdIns4P. The removal of PtdIns(4,5)P_2 disrupts actin dynamics, causing local detachment of the cortical cytoskeleton from the plasma membrane, which in turn leads to extensive membrane blebbing. Although IpgD and VPA0450 use the same molecular mechanism to facilitate internalization of the bacteria, the blebbing that is induced by VPA0450 has been shown to accelerate lysis[21–25]. This difference can be attributed to the strain-specific repertoires of effectors that are secreted by the bacterial pathogens. *V. parahaemolyticus* induces an orchestrated cell death using effectors that induce autophagy followed by blebbing and cell rounding, culminating in rapid cell lysis, thereby allowing the bacteria not only to evade phagocytosis but also to get access to the nutrients that are released from the lysed cells.

GTPase
A protein that cycles between the active, GTP-bound state and the inactive, GDP-bound state to regulate various cellular processes such as vesicle trafficking and actin dynamics. These enzymes are tightly regulated by GTPase-activating proteins and guanine nucleotide exchange factors.

Phosphatidylinositol
A small, negatively charged phospholipid molecule that is a key component of cell membranes and serves various roles in mediating signalling transduction.

Filopodia
Actin-rich cellular projections that aid in motility and environment sensing in eukaryotic cells.

Table 1 | **List of bacterial effectors targeting phosphoinositide metabolism and GTPases**

Pathogen	Effector	Activity	Substrate or target	Function	Refs
Phosphoinositide metabolism					
EPEC	Tir	Scaffold	SHIP2	Induces actin pedestal formation	26
Legionella pneumophila	SidC and SdcA	Adaptors	PtdIns4P	Recruit ER membranes to form the LCV	32,33
	SidM	Adaptor	PtdIns4P	Recruits ER membranes to form the LCV	36
Mycobacterium tuberculosis	SapM	Phosphatase	PtdIns3P	Arrests phagosome maturation	29
	MptpB	Phosphatase	PtdIns3P, PtdIns4P and PtdIns5P *in vitro*	Arrests phagosome maturation	30
S. Typhimurium	SopB	Phosphatase	PtdIns(4,5)P_2	Promotes membrane fission during bacterial internalization, reduces the surface charge of the SCV, inhibits the recruitment of RAB proteins to the SCV and arrests phagosome maturation	40–44
Shigella flexneri	IpgD	Phosphatase	PtdIns(4,5)P_2	Induces membrane blebbing and facilitates bacterial uptake	16
Vibrio parahaemolyticus	VPA0450	Phosphatase	PtdIns(4,5)P_2	Induces membrane blebbing, and contributes to cell lysis by interrupting plasma membrane integrity	21
GTPases					
EHEC	EspG	ARF binding	ARF GTPases	Disrupts GAP binding to ARF GTPases	77
EPEC	EspH	GEF binding	RHOGEFs	Disrupts GEF binding to RHO-family GTPases	75,76
L. pneumophila	LepB	RABGAP	RAB1 proteins	Hydrolyses RAB1•GTP	56
	SidM	RABGEF and AMPylation	RAB1 proteins	Recruits RAB1 proteins to the LCV by mimicking a RABGEF, and AMPylates RAB-family GTPases to reduce GAP activity	53–56
Pseudomonas aeruginosa	ExoS	RHOGAP	RHO and RAC proteins and CDC42	Induces actin reorganization and cell rounding	64,65
S. Typhimurium	SopE	RHOGEF	CDC42 and RAC1	Induces stress fibres and recruits RAB5 proteins to the SCV	51,59
		RABGEF	RAB5 proteins		
	SptP	RHOGAP	CDC42 and RAC1	Disrupts actin stress fibres	62
S. flexneri	IpgB1	RHOG mimic	ELMO–DOCK180 complex (a RAC1GEF)	Induces lamellipodia and membrane ruffling	70–72
	IpgB2	RHOAGEF	ROCKs and mDIA proteins	Induces stress fibres and membrane ruffling	74
V. parahaemolyticus	VopS	AMPylation	RHO and RAC proteins and CDC42	Disrupts downstream host effector binding, and induces actin reorganization and cell rounding	23,68
Yersinia pseudotuberculosis	YopE	RHOGAP	RHO and RAC proteins and CDC42	Induces actin reorganization and cell rounding	66,67

ARF, ADP-ribosylation factor; DOCK180, dedicator of cytokinesis 180 kDa; EHEC, enterhaemorrhagic *Escherichia coli*; ELMO, engulfment and cell motility; EPEC, enteropathogenic *E. coli*; ER, endoplasmic reticulum; ExoS, exoenzyme S; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; LCV, *Legionella*-containing vacuole; mDIA proteins, Diaphanous-related formins (also known as DRF or DIAPH proteins); PtdIns4P, phosphatidylinositol-4-phosphate; RABGAP, RAB-family-specific GAP; RHOGEF, RHO-family-specific GEF; ROCK, RHO-associated protein kinase; SHIP2, SH domain-containing inositol 5′-phosphate 2; SCV, *Salmonella*-containing vacuole; *S.* Typhimurium, *Salmonella enterica* subsp. *enterica* serovar Typhimurium.

EPEC is an extracellular pathogen that adheres to intestinal epithelial cells and forms F-actin-rich pedestals. Using its T3SS, EPEC translocates an intimin receptor, Tir, into the host cell by looping it through the plasma membrane, resulting in both its amino and carboxyl termini dangling in the cytoplasm[26]. The C terminus has sequence similarity with the cellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which contain two tyrosine residues (Tyr483 and Tyr511) that are responsible for recruiting SH2 domain-containing inositol 5′-phosphatase 2 (SHIP2), which converts PtdIns(3,4,5)P_3 to PtdIns(3,4)P_2 on the plasma membrane. The enrichment of PtdIns(3,4)P_2 recruits the adaptor protein lamellipodin (LPD; also known as RAPH1), an essential activator for actin pedestal formation, to this lipid platform[27]. The central domain of Tir, which is exposed on the cell surface, binds to the bacterial outer membrane protein intimin, and this leads to the clustering of intimin receptors, triggering a signalling cascade that regulates actin assembly at the plasma membrane[26].

Clearly, the type of phospholipids at the plasma membrane can dictate changes in the actin cytoskeleton. Bacterial pathogens manipulate the 'conversation' between the membrane and the actin cytoskeleton by changing the number and repertoire of phospholipids on the cytoplasmic side of the plasma membrane in an infected cell.

F-actin
The filamentous form of actin; a polymer of globular monomeric actin.

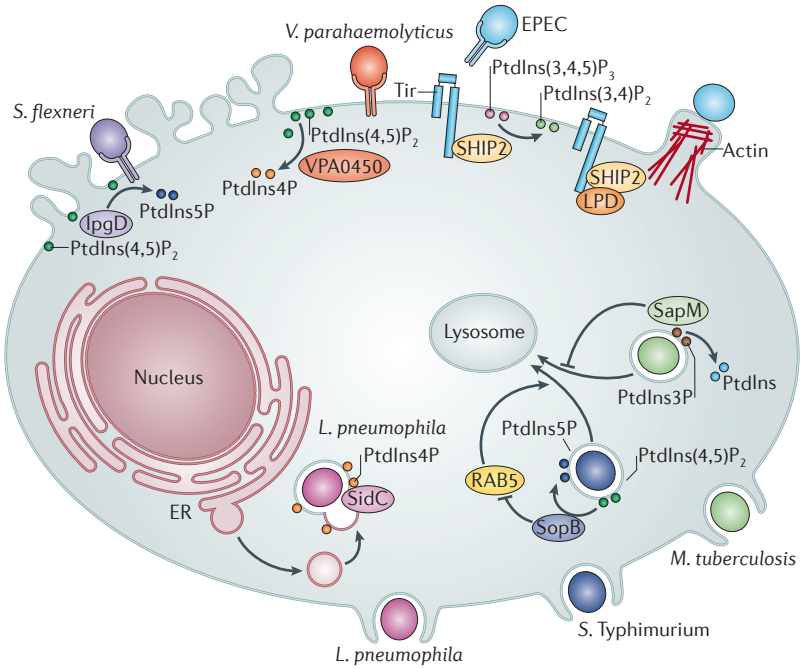

Figure 2 | **Bacterial effectors that modulate host phosphoinositide metabolism.** Bacterial effectors with phosphatase activity manipulate phosphoinositide (PI) levels at the host plasma membrane to disrupt actin dynamics at the cell cortex, thus altering membrane morphology. IpgD from *Shigella flexneri* and VPA0450 from *Vibrio parahaemolyticus* cause membrane blebbing. Some bacterial effectors exploit host phosphatases to alter PI homeostasis at the cell membrane. Tir from enteropathogenic *Escherichia coli* (EPEC) recruits SH domain-containing inositol-5′-phosphatase 2 (SHIP2), which enriches phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P_2) and thereby recruits lamellipodin (LPD) to form an actin pedestal at the bacterial attachment site. Intracellular pathogens use PI adaptor proteins to acquire the organellar membranes that are needed for establishing replicative vacuoles. SidC and SdcA from *Legionella pneumophila* anchor to PtdIns4P on the *Legionella*-containing vacuole (LCV) and recruit endoplasmic reticulum (ER) membranes to form an intracellular replicative niche for the pathogen. Bacterial effectors employ phosphatase activity to arrest maturation of the phagosomes that contain intracellular pathogens. SopB from *Salmonella enterica* subsp. *enterica* serovar Typhimurium reduces the level of PtdIns(4,5)P_2 on the *Salmonella*-containing vacuole (SCV), and this inhibits the recruitment of downstream RAB proteins, thereby blocking lysosomal fusion with the SCV. SapM from *Mycobacterium tuberculosis* hydrolyses PtdIns3P on the phagosomal membrane, inhibiting lysosomal fusion with the phagosome.

Blocking of phagosome maturation by intracellular pathogens. Phagocytosis is a defence mechanism that removes foreign particles such as bacteria. The process initiates with engulfment of the bacterium by a plasma membrane-derived intracellular vacuole termed a phagosome. This is followed by a series of membrane fusion events that massively reorganizes both the composition of phagosomal membranes and the phagosomal contents, a process known as phagosome maturation[28]. The pathway terminates with the formation of phagolysosomes, which kill the engulfed bacterium with low pH and digestive enzymes. Some bacteria have evolved strategies to escape from phagosomes or block phagosome maturation to avoid lysosomal digestion.

M. tuberculosis replicates in macrophage phagosomes and uses the bacterial phosphatase SapM to inhibit their maturation into phagolysosomes[29]. The amount of PtdIns3P on the phagosomal membrane is substantially lower for phagosomes harbouring live *M. tuberculosis* than for those containing dead bacteria[29], and this low level of PtdIns3P blocks phagosomes from fusing with late endosomes and lysosomes. The reduction of phagosomal PtdIns3P levels is attributed to SapM, which specifically dephosphorylates PtdIns3P, thereby arresting the phagosome maturation process. Another *M. tuberculosis* PI phosphatase, MptpB (also known as PtpB), exhibits broad substrate specificity towards PtdIns(3,5)P_2 and the PI monophosphates PtdIns3P, PtdIns4P and PtdIns5P *in vitro*[30], but the biologically relevant substrates for MptpB *in vivo* and its role in phagosome maturation are still undetermined.

L. pneumophila uses a specialized T4SS called defect in organelle trafficking/intracellular multiplication (Dot/Icm)[31]. This system is a conjugation apparatus that is required to form *Legionella*-containing vacuoles (LCVs) inside the host cells and to control the trafficking of these LCVs as they avoid the default endocytic maturation process[31]. LCVs are unique intracellular vacuolar compartments that are derived from host ER membranes and provide the replicative niche for *L. pneumophila*, protecting the bacteria from lysosomal degradation. Unlike the pathogens discussed above, *L. pneumophila* does not require bacterial or host phosphatase activity to achieve this subversion of host processes. Instead, *L. pneumophila* employs SidC and its paralogue SdcA, which are Dot/Icm-secreted proteins that bind to PtdIns4P on the LCV surface, leading to the recruitment of ER-derived vesicles[32,33]. The binding activity of SidC was mapped to a unique 20 kDa fragment that does not have sequence homology with any known eukaryotic PI-binding domain. This sequence, termed P4C (for PtdIns4P-binding of SidC), is suggested to function as a PtdIns4P-binding probe[33]. The LCV recruits vesicles that are secreted from ER exit sites[34]. The interaction of LCVs with early secretory vesicles and ER membranes is facilitated by SidC, as the acquisition of ER markers by LCVs is markedly decreased in a deletion mutant for both *sidC* and *sdcA*[32]. SidC also alters the membrane dynamics of LCVs and thereby inhibits their morphological transition from being tight to being spacious[32]. In the early stages of infection, the LCV membrane tightly associates with the surface of *L. pneumophila*. At later stages, on the acquisition of ER membrane, the vacuolar membrane detaches from the bacterium and expands, forming a spacious membrane structure[35]. LCVs in the Δ*sidC*Δ*sdcA* mutant do not undergo this morphological change and remain as tight vacuoles, which is an unfavourable environment for the bacterium to replicate in[32]. Therefore, SidC maintains LCV integrity by recruiting ER membranes and regulating the dynamics of LCV membranes. SidM (also known as DrrA), another Dot/Icm substrate that is secreted by *L. pneumophila*, can also bind to PtdIns4P on LCVs via a novel 10 kDa P4M (PtdIns4-binding of SidM(DrrA)) domain[36]. This is thought to lead to competition between SidC and SidM for binding to PtdIns4P on the LCV surface, as a *sidM*-mutant *L. pneumophila* exhibits higher levels of SidC on LCVs. Although the reason for this competition is not clear, both SidC and SidM promote the establishment of LCVs, thereby enabling bacterial replication.

Phagosome
A vacuole that is derived from the outer cell membrane of a host cell and that has engulfed a foreign particle.

Endoplasmic reticulum exit sites
Periphery regions of the endoplasmic reticulum where cargo proteins are exported in vesicles.

RAB GTPase
A member of the RAB family of small monomeric GTPases that are involved in the regulation of vesicle trafficking.

GTPase-activating proteins
(GAPs). A family of proteins that accelerate GTPase-mediated hydrolysis of GTP to GDP.

Guanine nucleotide exchange factors
(GEFs). A family of proteins that induce GTPases to exchange GTP for GDP, resulting in activation of the GTPases.

RHO
A family of small monomeric GTPases (including the RHO proteins, RAC proteins and CDC42) that are involved in the regulation of actin dynamics.

Guanine nucleotide dissociation inhibitor
(GDI). A protein that binds to a GDP-bound GTPase and holds it in an inactive, soluble state in the cytoplasm.

S. Typhimurium is a facultative intracellular pathogen that forms an intracellular compartment termed the SCV on entry into a host cell. The SCV interacts with the host endosomal trafficking pathway and undergoes fusions with early and late endosomes during maturation. However, *S.* Typhimurium regulates this trafficking using T3SS effectors to block lysosomal fusion with the SCV[37,38]. A recent study suggests that lysosome fusion does occur but is merely delayed in epithelial cells[39]. Clearly, SCV maturation is a complex process that might adopt different fates depending on the host cell type. SopB, a T3SS effector from *S.* Typhimurium, is a PI phosphatase that affects multiple processes during the course of infection, including bacterial invasion, SCV formation and SCV maturation[40–42]. SopB hydrolyses PtdIns(4,5)P_2 both at the plasma membrane and on the SCV membrane surface[41–44]. Decreased levels of PtdIns(4,5)P_2 at the plasma membrane promote membrane fission by reorganizing the actin cytoskeleton during bacterial internalization[41,44]. SopB also mediates the production and maintenance of high levels of PtdIns3P on the SCV surface through an indirect effect of its phosphatase activity: SopB recruits the RAB GTPase RAB5 proteins (hereafter referred to as RAB5) and the RAB5 effector VPS34 (also known as PIK3C3), a PtdIns 3-kinase that generates PtdIns3P, to the SCV through a process that is dependent on the reduction of PtdIns(4,5)P_2 (REF. 42). It has been shown that a decrease in the levels of PtdIns(4,5)P_2 and phosphatidylserine (PS), a negatively charged phospholipid, that is mediated by SopB reduces the negative charge of the SCV membrane surface. This in turn hinders the recruitment of RAB35 and RAB23, which normally associate with the membrane through their cationic prenyl tails, to SCVs, thereby inhibiting phagosome–lysosome fusion[42]. Overall, manipulation of the SCV lipid composition by SopB alters the recruitment of RAB GTPases to help SCVs avoid lysosomal degradation and persist in the host cells, thereby creating a replicative niche for *S.* Typhimurium. Manipulation of the membrane surface charge of the bacterium-containing vacuole could exert a global effect on the interaction of this vacuole with host proteins that rely on electrostatic interactions. Therefore, it was speculated to be a general mechanism that is adopted by a wide range of intracellular pathogens to manipulate the fate of these vacuoles by depleting them of negatively charged PIs.

PIs often act in concert with small GTPases to recruit cytosolic proteins to host membranes. This allows PIs and small GTPases to exert regulatory control on each other[9]. PIs can bind and activate GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), whereas GTPases control PI metabolism by regulating PI kinases and PI phosphatases[9]. Manipulation of this close functional interplay between PI metabolism and GTPase signalling can be observed in many bacterial infections. The next section reviews the bacterial subversion of host GTPase signalling.

GTPase signalling and endosomal trafficking

Proteins that hydrolyse GTP to GDP, called GTPases or G proteins, use this hydrolysis to serve a multitude of functions in the eukaryotic cell; for example, these proteins are involved in actin dynamics, vesicle trafficking, phagocytosis, cell growth and cell differentiation. GTPases can be divided into two subclasses: the heterotrimeric G proteins and the small monomeric GTPases. The RAS superfamily of small GTPases is involved in a diverse range of cellular processes and consists of several subfamilies, including the RAB, RHO, ADP-ribosylation factor (ARF), RAN and RAS families. In this section, we focus on bacterial effectors that target RAB, ARF and RHO GTPases (FIG. 3; TABLE 1) (for reviews, see REFS 45–48).

By cycling between their inactive, GDP-bound form and their active, GTP-bound form, GTPases function as a molecular switch. In the GTP-bound form, the GTPase is in a conformational 'on' state and can interact with downstream partners (also termed effectors). In the GDP-bound form, the GTPase is in a conformational 'off' state and can no longer bind the downstream effectors. This cycle is facilitated by two classes of regulatory proteins: GAPs and GEFs. GAPs turn the GTPase 'off' by accelerating the intrinsic rate of GTP hydrolysis, resulting in the formation of GDP and phosphate. By contrast, GEFs turn the switch 'on' by facilitating the dissociation of GDP and allowing the more abundant GTP to bind. A third factor, the guanine nucleotide dissociation inhibitor (GDI), can bind the GDP-bound GTPase in the cytosol and sequester it from its site of action, the membrane. Membrane localization of a GTPase is determined by the post-translational addition of a lipid moiety such as a geranylgeranyl or myristoyl group to a target sequence at the C terminus of the protein.

Box 1 | **Phosphoinositide homeostasis in the cell**

Phosphoinositides (PIs) are crucial components of cell membranes and have important roles in various cellular processes, such as membrane dynamics, actin polymerization and vesicle trafficking. Their steady-state and differential membrane distributions are strictly controlled by membrane-localized PI kinases and phosphatases, allowing tight spatial and temporal regulation of membrane-associated signalling.

- Phosphatidyinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) is mainly distributed to the plasma membrane, where it induces actin polymerization, which can modulate cell polarity and motility. Following phagocytosis of a bacterium, PtdIns(3,4,5)P_3 is enriched at the bacterial entry site.
- The majority of PtdIns(4,5)P_2 is found at the plasma membrane, where it regulates the organization of the actin cytoskeleton. PtdIns(4,5)P_2 is a precursor for inositol-1,4,5-trisphosphate and diacylglycerol (DAG), which are important secondary messengers for activating protein kinase C (PKC). Similarly to PtdIns(3,4,5)P_3, PtdIns(4,5)P_2 is found at sites of phagocytosis following bacterial infection.
- PtdIns(3,4)P_2 is enriched at the plasma membrane and in early endosomes.
- PtdIns(3,5)P_2 is mainly distributed to the late endosomes.
- PtdIns3P is enriched in the early endosomes and is involved in endocytic trafficking. It contributes to the recycling of membrane from the endosome to the plasma membrane, and to transport between the Golgi and the vacuole. It is also enriched in some bacterium-containing intracellular vacuoles during infection.
- PtdIns4P is the most abundant of the monophosphorylated PIs. It is mainly present in the Golgi membranes involved in anterograde membrane trafficking.
- PtdIns5P is the least characterized among all the PIs. It is proposed to be involved in the regulation of membrane transport from late endosomes to the plasma membrane.

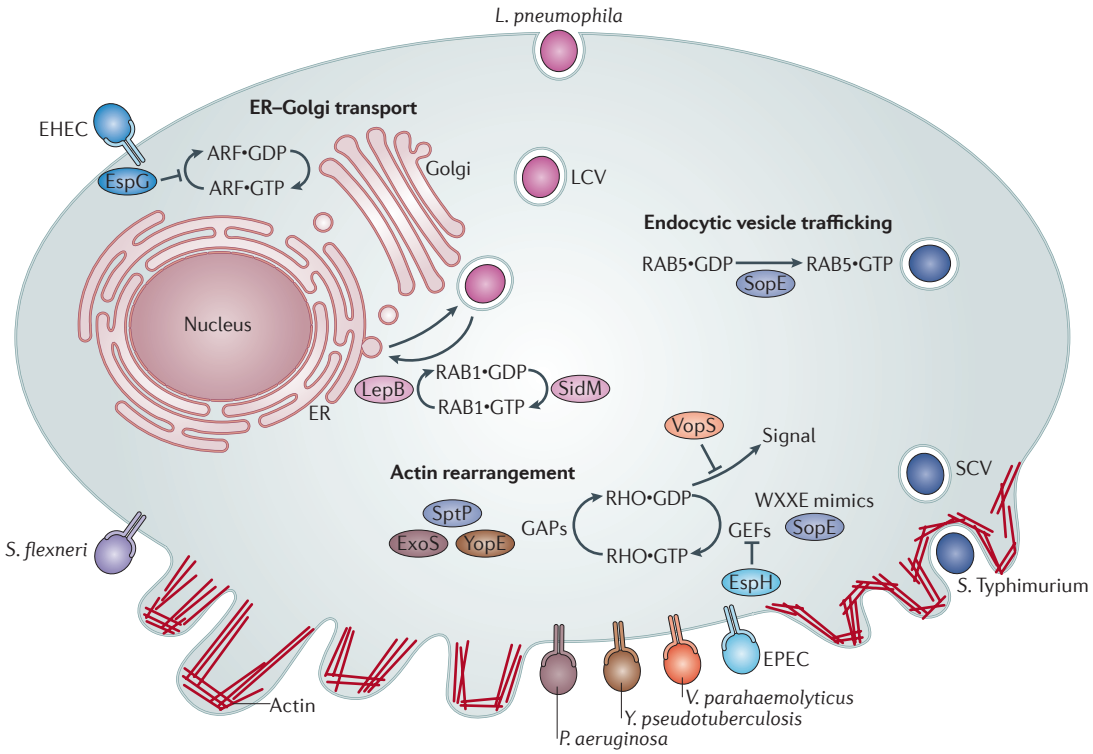

Figure 3 | **Bacterial effectors that target GTPase signalling pathways.** Bacterial effectors manipulate GTPases directly, by post-translational modification or by mimicking a guanine nucleotide exchange factor (GEF) or a GTPase-activating protein (GAP). On infection, pathogens use their effectors to target RHO-family GTPases in order to prevent phagocytosis and to alter actin-dependent membrane dynamics. EspG from enterohaemorrhagic *Escherichia coli* (EHEC) disrupts membrane trafficking by inhibiting the ADP-ribosylation factor (ARF)-family GTPases that regulate vesicle trafficking. Effectors also target RAB GTPases to alter membrane trafficking, prevent degradation by the lysosome and promote replication in the pathogen-containing vacuole. Bacterial effectors such as LepB and SidM from *Legionella pneumophila* act in opposition, whereas other effectors such as IpgB1 and IpgB2 (not shown) from *Shigella flexneri* act in concert to induce stress fibres and membrane ruffling. Bacterial effectors may also act as antagonists to increase infection efficiency and control different stages of infection: SopE from *Salmonella enterica* subsp. *enterica* serovar Typhimurium facilitates entry into the host cell, and SptP from the same pathogen facilitates replication in the host cell. See main text for details. EPEC, enteropathogenic *E. coli*; ER, endoplasmic reticulum; ExoS, exoenzyme S; LCV, *Legionella*-containing vacuole; *P. aeruginosa*, *Pseudomonas aeruginosa;* SCV, *Salmonella*-containing vacuole; *V. parahaemolyticus*, *Vibrio parahaemolyticus; Y. pseudotuberculosis*, *Yersinia pseudotuberculosis.*

The molecular switch of RAS-superfamily GTPases is regulated by two regions at the N terminus, switch I and switch II. These regions stabilize the γ-phosphate of GTP, as well as the Mg^{2+} ion that reduces the negative charge imparted by the phosphate groups on the guanine nucleotide. As seen in FIG. 3, bacterial effectors target and manipulate GTPases by tipping the balance between the 'on' and 'off' state, often by manipulating the switch regions[49].

Manipulation of RAB GTPases. RAB GTPases regulate multiple vesicular trafficking pathways such as the ER–Golgi pathway and the endosome–lysosome pathway. RAB proteins localize to intracellular compartments, where they cycle between the membrane-bound (active) and cytosolic (inactive) state. This cycling is aided by regulatory proteins such as RAB escort protein 1 (REP1; also known as CHM), which targets geranylated RAB proteins to the membrane, where a GEF replaces the GDP with GTP. Bacterial pathogens such as *S.* Typhimurium and *L. pneumophila* produce GEFs and GAPs that regulate RAB GTPases[50].

As mentioned above, SopB is a PI phosphatase of *S.* Typhimurium that alters the profile of the RAB GTPases that are recruited to the SCV, mainly through charge alteration and by acting as a platform for the recruitment of RAB5 (REF. 42). SopE, a RAB-specific GEF (RABGEF) mimic, recruits active RAB5 to the SCV by converting inactive RAB5•GDP to active RAB•GTP. Hence, SopE promotes fusion of the SCV with early endosomes and prevents its fusion with the lysosome[51,52].

SidM, the above-mentioned Dot/Icm-secreted effector from *L. pneumophila*, targets RAB1 proteins (hereafter referred to as RAB1), which are involved in ER–Golgi transport. SidM is a bifunctional enzyme: the C terminus functions as a RABGEF, whereas the N terminus catalyses AMPylation. As a RABGEF, SidM catalyses the exchange of GDP for GTP by changing the conformation of RAB1 residues that are important for nucleotide stabilization[53]. SidM AMPylates Tyr77 of the

AMPylation
A post-translational modification that involves the covalent attachment of AMP to a threonine or tyrosine residue on a protein substrate, resulting in an altered activity of the modified protein.

switch II region in RAB1, inhibiting GAP binding and, thus, GTP hydrolysis. AMPylation induces cell rounding and shrinkage, which contribute to the disruption of cell homeostasis and to cytotoxicity[54]. Inhibition of RAB protein activity retards intracellular growth of *L. pneumophila*[34]. Therefore, SidM is localized to the membrane through its interaction with PtdIns4P (see above) and uses a two-pronged approach to recruit RAB1•GTP to the LCV, mimicking a RABGEF and delaying GAP activity by AMPylation. In addition, SidM displaces the GDI that is bound to RAB1•GDP, therefore aiding in RAB1•GDP recruitment to the membrane for activation[55]. SidM-mediated RAB1 activation and recruitment to the LCV promote fusion of ER-derived vesicle with the LCV. Another *L. pneumophila* effector, LepB, then acts as a GAP for RAB1 (REF. 56), inactivating the GTPase to release it from the LCV and thus promote fusion of the LCV with the ER. In this way, *L. pneumophila* manipulates RAB1 in a sequential manner to promote maturation of the LCV and create a spacious replicative niche. During the initial phase of infection, *L. pneumophila* resides in the ER-derived vesicle that interacts with the secretory pathway; during the later stages of infection, when bacterial replication occurs, these vesicles acquire lysosomal markers[57].

Therefore, bacteria manipulate the enzymatic state of GTPases and their recruitment to membrane vesicles, resulting in the survival of intracellular pathogens.

Manipulation of RHO-family GTPases. RHO-family GTPases regulate different aspects of actin dynamics: activation of RHOA induces the formation of actin stress fibres; activation of RAC1 induces the formation of lamellipodia; and activation of CDC42 induces the formation of filopodia. Inactivation of RHO-family GTPases leads to a decrease in F-actin and increase in monomeric actin (G-actin), resulting in loss of cell shape, motility and ability to phagocytose or endocytose pathogens. All of these RHO-family proteins (RHOA, RAC1 and CDC42) are common targets of bacterial effectors.

S. Typhimurium manipulates RHO-family GTPases using the effectors SopE and SptP. SopE acts as a GEF for CDC42 and RAC1 , whereas SptP acts as a GAP for CDC42 and RAC1 (REF. 58). SopE is translocated into the cell to induce actin rearrangement and membrane ruffling to facilitate entry of the pathogen into the cell and formation of SCVs; SptP then disrupts these actin filaments to restore actin organization in the cell[59]. Although these two enzymes are structurally different, they function similarly to the eukaryotic GEFs and GAPs, respectively, but have a higher catalytic efficiency than their eukaryotic counterparts[60]. SptP is a bifunctional enzyme: it functions as both a GAP and a tyrosine phosphatase[60–62]. It disrupts the actin cytoskeleton by binding to RAC1 and catalysing GTP–GDP exchange. The antagonistic effectors SopE and SptP are coordinately regulated: SopE acts first, with a short half-life, to accelerate uptake of the pathogen. After SopE's degradation, SptP takes over and dominates the disasssembly of F-actin, thereby crippling vacuolar migration to the lysosome and ensuring a safe haven for replication of the pathogen in the vesicle[63].

The GAP domain of SptP is similar to a region in each of the *Pseudomonas aeruginosa* effector exoenzyme S (ExoS) and the *Yersinia pseudotuberculosis* effector YopE. ExoS is also a bifunctional enzyme: the C terminus has ADP-ribosyltransferase activity, and the N terminus has the GAP activity. However, the GAP activity is lessened by auto-ADP-ribosylation at Arg146, which is necessary for GTP hydrolysis[64]. ExoS targets RHOA, RAC1 and CDC42 to disrupt the actin cytoskeleton and induce cell rounding[65]. Similarly, YopE is a RHOGAP that induces depolymerization of actin, cell rounding and cytotoxicity in HeLa cells[66]. The GAP activity of YopE is also essential for the antiphagocytic function of this protein[67]. In contrast to mimicking GAP activity, effectors may also inactivate GTPases by modifying them post-translationally. VopS from *V. parahaemolyticus* is a T3SS effector that targets RHO-family GTPases, AMPylating the Thr35 residue in the switch I region that is involved in substrate binding. AMPylation disrupts RHO-family GTPase binding to downstream effectors such as the PAK proteins[23]. Transfection of VopS also induces cell rounding via AMPylation and inactivation of RAC1, RHOA and CDC42 (REF. 68). Cell rounding benefits the pathogen because the cells are antiphagocytic as a result of the actin cytoskeleton disruption.

By contrast, effectors may manipulate the actin cytoskeleton to induce filopodia formation or membrane ruffling, thus drastically changing membrane morphology. The host cell engulfment and cell motility (ELMO)–dedicator of cytokinesis 180 kDa (DOCK180) complex (consisting of any one of three ELMO variants and any one of the DOCK180-related proteins) is activated by RHOG, which localizes this complex to the membrane, where it activates RAC1. IpgB1, a T3SS effector of *S. flexneri*, acts as a RHOG mimic, binding to the ELMO–DOCK180 complex and activating RAC1, which then induces lamellipodia and membrane ruffling[69–73]. As a result, IpgB1 increases infection efficiency. Another *S. flexneri* effector, IpgB2, induces membrane ruffling and stress fibres by mimicking a RHOAGEF[74]. Both EPEC and enterohaemorrhagic *E. coli* (EHEC) also manipulate the actin cytoskeleton, but each by a unique mechanism. EspH, a T3SS effector from EPEC, is a membrane-associated protein that represses filopodia and induces pedestal formation. EspH binds to the RHOGEF and disrupts its binding to RHO-family GTPases, thereby causing filament disassembly. By manipulating actin dynamics, EPEC uses this unique GEF-binding effector to prevent phagocytosis[75,76]. EspG is an effector protein that is translocated into the host cell by EHEC to induce fragmentation of the Golgi apparatus and disrupt membrane trafficking. EspG targets ARF-family GTPases that regulate vesicle trafficking. The effector binds to the switch I region and the nucleotide-binding pocket of ARF-family GTPases to inhibit the interaction between the ARFGAP and the GTPase by steric hindrance. Furthermore, EspG acts as a novel bacterial catalytic scaffold that links the inhibition of ARF family GTPases with the stimulation of PAK proteins to manipulate the host cell membrane[77].

Stress fibres
Bundles of actin filaments.

Lamellipodia
Dynamic actin-rich regions on the edge of a cell that aid in cell motility.

Autophagosome
A double-membraned compartment that contains host cytoplasm and organelles and is formed in cells undergoing autophagy.

Bacterial effectors use a wide range of strategies to enter the host cell, prevent phagocytosis and disrupt host cell signalling. RAB- and RHO-family GTPases are obvious targets owing to their roles in vesicle trafficking and actin dynamics, respectively. Another important target in vesicle trafficking and host defence is the autophagy pathway. Although host cells use autophagy to degrade pathogens, several pathogens exploit this pathway to replicate and infect[78] (FIG. 4; TABLE 2), as discussed in the next section.

Membrane dynamics and autophagy

Autophagy is a process by which cells degrade and recycle cellular contents. It is triggered to protect cells as they undergo various stress conditions, including starvation, lack of growth factors, oxidative stress and the accumulation of protein aggregates (for a review, see REF. 79). Autophagy begins with the formation of a double-membraned structure called an autophagosome, which encapsulates part of the cytoplasm or organelles. The autophagosome then fuses with the lysosome to form the autolysosome, the contents of which are degraded and used as nutrients for the cell. Autophagy is also used as a cellular defence mechanism against the invasion of pathogenic bacteria, as the vacuoles containing these pathogens can fuse with autophagosomes and deliver the pathogens to lysosomes. For example, intracellular vacuoles containing *M. tuberculosis* undergo phagolysosomal maturation and elimination from the host on the induction of autophagy[80]. *L. monocytogenes*, an intracellular pathogen that escapes from phagosomes and persists in the cytoplasm, can be internalized by autophagic vacuoles and degraded by lysosomal fusion[81]. However, some pathogens have evolved strategies to subvert autophagy to their own advantage by establishing the autophagosome as their replicative niche (FIG. 4) (for reviews, see REFS 82–85). This niche not only masks them from host defence mechanisms but also may provide nutrients for the bacteria to survive and grow.

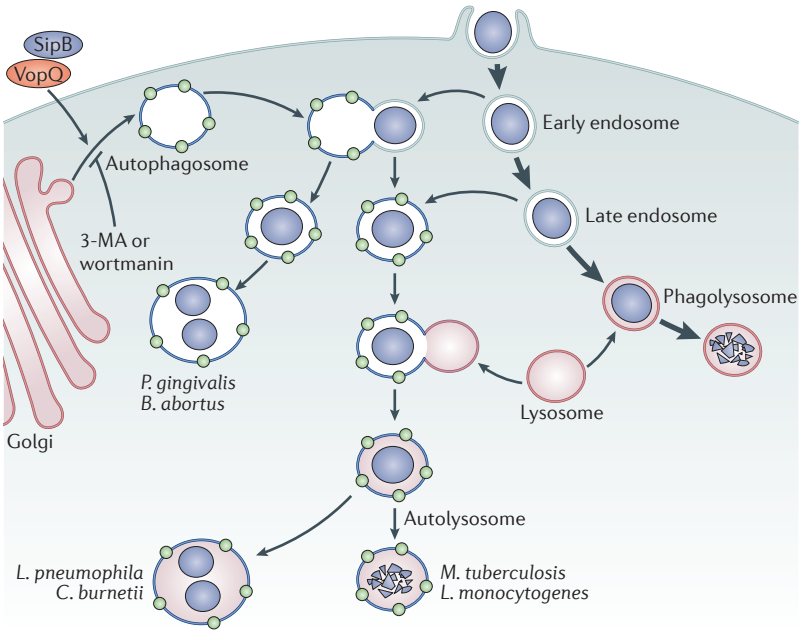

Figure 4 | **Bacterial pathogens that subvert the autophagic pathway.** On infection with *Mycobacterium tuberculosis* and *Listeria monocytogenes*, the host cells undergo autophagy to engulf the bacteria into autophagosomes and degrade them by fusion with the lysosome. However, some intracellular bacterial pathogens subvert the autophagic pathway by using autophagosomes as their replicative niche. For example, *Porphyromonas gingivalis* and *Brucella abortus* replicate in autophagosomes that evade lysosomal fusion. Conversely, *Legionella pneumophila* and *Coxiella burnetii* manage to survive in acidic autophagic vacuoles that acquire lysosomal markers. The survival of bacteria using autophagosomes is compromised when host cells are treated with the autophagy inhibitors 3-methyladenine (3-MA) and wortmannin because the bacteria can no longer prevent normal phagosome maturation (indicated by bold arrows). The *Salmonella enterica* subsp. *enterica* serovar Typhimurium type III secretion system (T3SS) effector SipB, which localizes to and disrupts mitochondria, and the *Vibrio parahaemolyticus* T3SS effector VopQ are thought to induce autophagy, and this allows the bacteria to access nutrients after host cell lysis.

Interrupting the maturation of autophagosomes. *Porphyromonas gingivalis*, a periodontal pathogen that is also associated with atherosclerosis and cardiovascular disease, resides in an intracellular vacuole that has characteristics of early and late autophagosomes: the vacuoles carry ER markers early after pathogen internalization, followed by late-endosomal and lysosomal markers[86]. On treatment with autophagy inhibitors 3-methyladenine (an inhibitor of PI 3-kinases) and wortmannin, the bacterium-containing vacuoles undergo massive changes in their composition, as shown using membrane markers. The inhibitors block the initial formation of autophagosome-like structures, so the vacuoles in treated cells exhibit the characteristics of lysosomes rather than of autophagosomes, resulting in a marked decrease in bacterial survival. This supports the hypothesis that the induction of autophagy is required for *P. gingivalis* to avoid lysosomal degradation and persist in the host cells.

Brucella abortus also replicates in intracellular compartments resembling autophagosomes in HeLa cells[87,88]. These compartments sequentially acquire markers for early-endosomal and ER membranes but do not harbour the lysosomal protease cathepsin D in the later stage of phagosome maturation, indicating that these compartments escape from lysosomal fusion. As was shown for *P. gingivalis*, treatment with autophagy inhibitors reduces the survival of intracellular *B. abortus*. The biogenesis of autophagosome-like vacuoles in *B. abortus* has been shown to depend on the *virB* operon, which encodes genes that are homologous to those associated with the T4SS in other species. *virB* mutants fail to avoid autophagolysosome formation[89]. This underscores the importance of the *virB* operon for maintaining an early-autophagosome-like compartment.

L. pneumophila replicates in LCVs that resemble nascent autophagosomes, being composed of two or more layers of ER-derived membranes[90]. Induction of the host autophagy pathway by amino acid starvation in macrophages increased intracellular bacterial growth and bacterial association with the ER[90]. As for other pathogens that use the autophagy pathway, inhibition of the autophagy pathway with 3-methyladenine increased *L. pneumophila* degradation[91]. Although the LCV fuses

Table 2 | **Bacterial pathogens that exploit host autophagic pathways**

Pathogen	Effector	Vesicle markers	Comments	Refs
Brucella abortus	Unknown	LAMP1, SEC61B and MDC	• *virB* is required for autophagosome formation • Inhibition of autophagy reduces bacterial survival	87–89
Coxiella burnetii	Unknown	LC3, MDC, RAB7 proteins and LysoTracker	• The bacterium replicates within an acidified autophago-lysosome-like vacuole • Inhibition of autophagy reduces bacterial survival • Induction of autophagy favors bacteria infection and replication	94,95
Legionella pneumophila	Unknown	LAMP1, MDC, RAB7 proteins, RAB14, ARF1, ATG7*, LC3 and cathepsin D	• The bacterium replicates within an acidified autophago-lysosome-like vacuole • Inhibition of autophagy reduces bacterial survival • Induction of autophagy favors bacterial infection and replication	90,91
Porphyromonas gingivalis	Unknown	RAB5 proteins and ATG7* (early vesicles); BIP and LAMP1 (late vesicles)	• The bacterium stimulates autophagosome formation • Inhibition of autophagy reduces bacterial survival	86
S. Typhimurium	SipB	SEC61 and Mytotracker	• The bacterium induces autophagosome formation • SipB deregulates mitochondrial intergrity and causes autophagy	96
Vibrio parahaemolyticus	VopQ	LC3	• VopQ is necessary and sufficient to induce autophagy during infection	22

ARF, ADP-ribosylation factor 1; MDC, monodansylcadaverine; S. Typhimurium, *Salmonella enterica* subsp. *enterica* serovar Typhimurium. *Previously known as HsGsa7p.

with the lysosome later during the course of infection (the LCV acquires lysosomal markers, such as LAMP1 and cathepsin D), *L. pneumophila* manages to replicate in this acidic vacuole by slowing down the maturation of the autophagosome[57]. Despite this view of *L. pneumophila* usurping the autophagic pathway as a strategy to survive inside the host cell, a recent study of the intracellular replication of *L. pneumophila*, using the social amoeba *Dictyostelium discoideum* (a well-established model for studying host–pathogen interactions), suggested that autophagy is dispensable for bacterial replication[92]. Mutations of the *apg* (also known as *atg*) genes that are essential for the formation of autophagosomes in the amoeba (*apg1*, *apg5*, *apg6*, *apg7* and *apg8*) did not impair the replication of *L. pneumophila* or the morphology of the LCV[92]. However, it is unclear whether autophagy is induced by the host or the pathogen in this infection model. Another study showed that disruption of *D. discoideum atg9*, which encodes an important component of the core autophagy machinery, reduced the clearance of *L. pneumophila* and enhanced pathogen replication[93]. These discoveries demonstrate that autophagy is used by bacterial pathogens for establishing a replicative niche and by the host cells as a defence mechanism against bacteria.

Coxiella burnetii also replicates in acidified intracellular vacuoles that can be labelled with LysoTracker as well as with markers for autophagic vesicles, such as monodansylcadaverine (MDC) and LC3 (REF. 94). The addition of autophagy inhibitors blocks the formation of *Coxiella*-containing phagolysosome-like vacuoles. Moreover, the induction of autophagy favours *C. burnetii* infection and replication in Chinese hamster ovary (CHO) cells, implying that there is an interaction between the pathogen and the autophagic pathway[95]. However, the specific effectors that are involved in subverting autophagic pathways have not been determined.

Pathogens that induce autophagy. SipB, a T3SS effector from *S.* Typhimurium, is suspected to induce autophagy on infection of macrophages. *S.* Typhimurium causes the formation of multimembraned intracellular structures that are similar to autophagosomes, containing both mitochondrial and ER markers[96]. SipB localizes to mitochondria and disorders their structure, a characteristic that is not observed with a *sipB* mutant strain despite the bacterial localization being the same as for a wild-type strain. This suggests that SipB deregulates mitochondrial integrity, leading to the induction of autophagy and cell death.

VopQ is a *V. parahaemolyticus* T3SS effector that has no homology to any protein with a known function but is capable of inducing autophagy within an hour of *V. parahaemolyticus* infection of HeLa cells[22]. It has been shown that VopQ is necessary and sufficient to induce autophagy and that VopQ-induced autophagy does not proceed through nutrient deprivation, mTOR signalling or PI 3-kinase signalling pathways[22]. Macrophages that are infected with *V. parahaemolyticus* exhibit autophagic vesicles and cannot phagocytose the bacterium. However, when macrophages are infected with a *V. parahaemolyticus* strain lacking VopQ, the host cell can phagocytose the bacterium. Therefore, VopQ is likely to be instrumental in rearranging intracellular membranes and preventing phagocytosis of the bacteria by the host cell.

Autophagy is a cellular adaptation for coping with stress and also an antimicrobial defence mechanism during pathogenesis. However, many bacterial pathogens have evolved to manipulate autophagy for their own benefit, either by directly inducing it or by subverting the pathway that has been activated on invasion. For intracellular bacteria, the key strategy is to usurp the autophagosomes for replication but avoid lysosomal fusion. This is an efficient way for pathogens to benefit from their interaction with the host membrane, because these compartments

LC3
(Microtubule-associated protein light chain 3). The cytosolic form of this protein, LC3-I, is lipidated and conjugated to phosphatidylethanolamine to form LC3-II, which then localizes to autophagosomal membranes. The increase in the conversion of LC3-I to LC3-II can be monitored as a marker for the induction of autophagy.

not only protect the bacteria from degradative enzymes and immune responses, but also provide nutrients from cellular debris. To date, *V. parahaemolyticus* is the only extracellular pathogen known to induce autophagy by delivering a dedicated effector into the host cell. It is likely that these bacteria have adopted the strategy to evade phagocytosis as well as to obtain nutrients.

Others mechanisms to alter membrane dynamics

Bacterial effectors interact with host membranes via several other mechanisms. SifA, a T3SS effector from *S.* Typhimurium, induces the formation of Sifs, which are tubular filaments that extend from the SCV. SifA moves along microtubules from the SCV to form Sifs, controlling endocytic trafficking of the SCV along microtubules and maintaining the integrity of SCVs[5–7,97]. *sifA* mutant bacteria lose the stability of SCVs and are released to the host cytosol, rendering them unable to replicate[97]. SipA, an effector that is involved in initial bacterial uptake, persists on the cytosolic face of SCVs after bacterial entry and cooperates with SifA to achieve SCV maturation and perinuclear positioning[98]. Another effector that is known to regulate the membrane dynamics of SCVs is SopD2 (REF. 99). This protein has an antagonistic role to SifA and destabilizes the SCV; a Δ*sifA*Δ*sopD2* double-mutant strain rescued the reduction of bacteria in SCVs that was observed with the *sifA* mutant strain[99]. The Δ*sifA*Δ*sopD2* double mutant also produces a filamentous structure that is distinct from Sifs (as it does not contain the lysosomal glycoprotein LAMP1, which is a characteristic of Sifs), termed a LAMP1-negative tubule (LNT). Therefore, SopD2 inhibits the vesicular trafficking of the SCV and its formation of the LNT.

CagA, a T4SS protein from the oncogenic bacterium *Helicobacter pylori* (a pathogen that can lead to gastric carcinoma), interacts with PS on the host membrane to promote bacterial entry[100]. CagA induces the redistribution of PS to the outer leaflet of the plasma membrane at the *H. pylori* attachment site. CagA then physically interacts with PS to initiate the internalization of the bacterium by endocytosis. On entering polarized epithelial cells, the CagA–PS interaction localizes CagA to the inner leaflet of the plasma membrane, where it recruits host proteinase-activated receptor 1 (PAR1)–MARK family kinases, resulting in junctional and polarity defects of the host cell. Therefore, the interaction between CagA and host membrane PS regulates the entry of the bacterium and the subsequent localization and pathophysiological action of CagA.

Concluding remarks

Bacterial pathogens interact with host cell membranes to trigger a wide range of changes in the host cell during the course of infection, including altered dynamics between the plasma membrane and the actin cytoskeleton, and subversion of membrane-associated pathways that are involved in vesicle trafficking. PI metabolism, GTPase signalling and the autophagic pathway are often targeted by pathogens to damage or exploit host cells for pathogen survival.

Although bacterial effectors may manipulate multiple organelles such as the mitochondria and the nucleus, the cell membrane is the initial target of many pathogens. Manipulation of the cell membrane can affect many events, including actin remodelling, phagocytosis, entry into host cells and replication in host cells. Functional characterization of known effectors as well as identification of novel effectors involved in such processes will undoubtedly lead to new discoveries. GTPase signalling and autophagy have roles in many diseases such as cancer and neurological disorders[79,101]. Although several effectors that target GTPases have been characterized, many of their downstream signalling pathways have yet to be elucidated. By contrast, few bacterial effectors involved in subverting autophagy are known, as the field is relatively new. In terms of manipulation of host PI metabolism, physiological substrates of some of the known bacterial phosphatases and their *in vivo* products are yet to be determined. Moreover, work uncovering the specific endocytic pathways that are targeted by bacterial phosphatases or PI adaptor proteins is still in its infancy. The discovery and characterization of the effectors that modulate these pathways during infection will not only enhance our understanding of the interaction between host and pathogen, but also provide insights into fundamental aspects of eukaryotic signalling pathways.

1. Anderson, D. M. & Schneewind, O. Type III machines of Gram-negative pathogens: injecting virulence factors into host cells and more. *Curr. Opin. Microbiol.* **2**, 18–24 (1999).
2. Cascales, E. & Christie, P. J. The versatile bacterial type IV secretion systems. *Nature Rev. Microbiol.* **1**, 137–149 (2003).
3. Backert, S. & Meyer, T. F. Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr. Opin. Microbiol.* **9**, 207–217 (2006).
4. Cossart, P. & Sansonetti, P. J. Bacterial invasion: the paradigms of enteroinvasive pathogens. *Science* **304**, 242–248 (2004).
5. Garcia-del Portillo, F., Zwick, M. B., Leung, K. Y. & Finlay, B. B. *Salmonella* induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells. *Proc. Natl Acad. Sci. USA* **90**, 10544–10548 (1993).
6. Stein, M. A., Leung, K. Y., Zwick, M., Garcia-del Portillo, F. & Finlay, B. B. Identification of a *Salmonella* virulence gene required for formation of filamentous structures containing lysosomal membrane glycoproteins within epithelial cells. *Mol. Microbiol.* **20**, 151–164 (1996).
7. Brumell, J. H., Goosney, D. L. & Finlay, B. B. SifA, a type III secreted effector of *Salmonella typhimurium*, directs *Salmonella*-induced filament (Sif) formation along microtubules. *Traffic* **3**, 407–415 (2002).
8. Drecktrah, D. *et al.* Dynamic behavior of *Salmonella*-induced membrane tubules in epithelial cells. *Traffic* **9**, 2117–2129 (2008).
9. Di Paolo, G. & De Camilli, P. Phosphoinositides in cell regulation and membrane dynamics. *Nature* **443**, 651–657 (2006).
10. Payrastre, B. *et al.* Phosphoinositides: key players in cell signalling, in time and space. *Cell Signal.* **13**, 377–387 (2001).
11. Saarikangas, J., Zhao, H. & Lappalainen, P. Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides. *Physiol. Rev.* **90**, 259–289 (2010).
12. Pizarro-Cerda, J. & Cossart, P. Subversion of phosphoinositide metabolism by intracellular bacterial pathogens. *Nature Cell Biol.* **6**, 1026–1033 (2004).
13. Hilbi, H. Modulation of phosphoinositide metabolism by pathogenic bacteria. *Cell. Microbiol.* **8**, 1697–1706 (2006).
14. Weber, S. S., Ragaz, C. & Hilbi, H. Pathogen trafficking pathways and host phosphoinositide metabolism. *Mol. Microbiol.* **71**, 1341–1352 (2009).
15. Niebuhr, K. *et al.* IpgD, a protein secreted by the type III secretion machinery of *Shigella flexneri*, is chaperoned by IpgE and implicated in entry focus formation. *Mol. Microbiol.* **38**, 8–19 (2000).
16. Niebuhr, K. *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**, 5069–5078 (2002).
17. Raucher, D. *et al.* Phosphatidylinositol 4,5-bisphosphate functions as a second messenger that regulates cytoskeleton–plasma membrane adhesion. *Cell* **100**, 221–228 (2000).
18. Charras, G. T., Coughlin, M., Mitchison, T. J. & Mahadevan, L. Life and times of a cellular bleb. *Biophys. J.* **94**, 1836–1853 (2008).

19. Charras, G. & Paluch, E. Blebs lead the way: how to migrate without lamellipodia. *Nature Rev. Mol. Cell Biol.* **9**, 730–736 (2008).
20. Charras, G. T. A short history of blebbing. *J. Microsc.* **231**, 466–478 (2008).
21. Broberg, C. A., Zhang, L., Gonzalez, H., Laskowski-Arce, M. A. & Orth, K. A *Vibrio* effector protein is an inositol phosphatase and disrupts host cell membrane integrity. *Science* **329**, 1660–1662 (2010).
Demonstrates a mechanism of action for an effector from *V. parahaemolyticus* that manipulates plasma membrane integrity via PI phosphatase activity.
22. Burdette, D. L., Seemann, J. & Orth, K. *Vibrio* VopQ induces PI3-kinase-independent autophagy and antagonizes phagocytosis. *Mol. Microbiol.* **73**, 639–649 (2009).
23. Yarbrough, M. L. *et al.* AMPylation of Rho GTPases by *Vibrio* VopS disrupts effector binding and downstream signaling. *Science* **323**, 269–272 (2009).
Describes a bacterial effector that catalyses a novel post-translational modification, AMPylation, to modify RHO-family GTPases and disrupt the actin cytoskeleton.
24. Burdette, D. L., Yarbrough, M. L. & Orth, K. Not without cause: *Vibrio parahaemolyticus* induces acute autophagy and cell death. *Autophagy* **5**, 100–102 (2009).
25. Burdette, D. L., Yarbrough, M. L., Orvedahl, A., Gilpin, C. J. & Orth, K. *Vibrio parahaemolyticus* orchestrates a multifaceted host cell infection by induction of autophagy, cell rounding, and then cell lysis. *Proc. Natl Acad. Sci. USA* **105**, 12497–12502 (2008).
26. Hayward, R. D., Leong, J. M., Koronakis, V. & Campellone, K. G. Exploiting pathogenic *Escherichia coli* to model transmembrane receptor signalling. *Nature Rev. Microbiol.* **4**, 358–370 (2006).
27. Smith, K., Humphreys, D., Hume, P. J. & Koronakis, V. Enteropathogenic *Escherichia coli* recruits the cellular inositol phosphatase SHIP2 to regulate actin-pedestal formation. *Cell Host Microbe* **7**, 13–24 (2010).
Identifies the critical residues of the EPEC protein Tir that are essential for recruitment of host SHIP2, which (through scaffolding and phosphatase activity) regulates actin pedestal formation.
28. Vieira, O. V., Botelho, R. J. & Grinstein, S. Phagosome maturation: aging gracefully. *Biochem. J.* **366**, 689–704 (2002).
29. Vergne, I. *et al.* Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. *Proc. Natl Acad. Sci. USA* **102**, 4033–4038 (2005).
30. Beresford, N. *et al.* MptpB, a virulence factor from *Mycobacterium tuberculosis*, exhibits triple-specificity phosphatase activity. *Biochem. J.* **406**, 13–18 (2007).
31. Hubber, A. & Roy, C. R. Modulation of host cell function by *Legionella pneumophila* type IV effectors. *Annu. Rev. Cell Dev. Biol.* **26**, 261–283 (2010).
32. Ragaz, C. *et al.* The *Legionella pneumophila* phosphatidylinositol-4 phosphate-binding type IV substrate SidC recruits endoplasmic reticulum vesicles to a replication-permissive vacuole. *Cell. Microbiol.* **10**, 2416–2433 (2008).
33. Weber, S. S., Ragaz, C., Reus, K., Nyfeler, Y. & Hilbi, H. *Legionella pneumophila* exploits PI(4)P to anchor secreted effector proteins to the replicative vacuole. *PLoS Pathog.* **2**, e46 (2006).
34. Kagan, J. C. & Roy, C. R. *Legionella* phagosomes intercept vesicular traffic from endoplasmic reticulum exit sites. *Nature Cell Biol.* **4**, 945–954 (2002).
35. Li, Z., Solomon, J. M. & Isberg, R. R. *Dictyostelium discoideum* strains lacking the RtoA protein are defective for maturation of the *Legionella pneumophila* replication vacuole. *Cell. Microbiol.* **7**, 431–442 (2005).
36. Brombacher, E. *et al.* Rab1 guanine nucleotide exchange factor SidM is a major phosphatidylinositol 4-phosphate-binding effector protein of *Legionella pneumophila*. *J. Biol. Chem.* **284**, 4846–4856 (2009).
Identifies the RAB1GEF from *L. pneumophila*, SidM, as a predominant PtdIns4P-binding protein, and maps the specific binding domain that is essential for anchoring SidM to PtdIns4P on LCVs.
37. Buchmeier, N. A. & Heffron, F. Inhibition of macrophage phagosome-lysosome fusion by *Salmonella typhimurium*. *Infect. Immun.* **59**, 2232–2238 (1991).
38. Garvis, S. G., Beuzon, C. R. & Holden, D. W. A role for the PhoP/Q regulon in inhibition of fusion between lysosomes and *Salmonella*-containing vacuoles in macrophages. *Cell. Microbiol.* **3**, 731–744 (2001).
39. Brumell, J. H., Tang, P., Mills, S. D. & Finlay, B. B. Characterization of *Salmonella*-induced filaments (Sifs) reveals a delayed interaction between *Salmonella*-containing vacuoles and late endocytic compartments. *Traffic* **2**, 643–653 (2001).
40. Hernandez, L. D., Hueffer, K., Wenk, M. R. & Galan, J. E. *Salmonella* modulates vesicular traffic by altering phosphoinositide metabolism. *Science* **304**, 1805–1807 (2004).
41. Terebiznik, M. R. *et al.* Elimination of host cell PtdIns(4,5)P_2 by bacterial SigD promotes membrane fission during invasion by *Salmonella*. *Nature Cell Biol.* **4**, 766–773 (2002).
42. Mallo, G. V. *et al.* SopB promotes phosphatidylinositol 3-phosphate formation on *Salmonella* vacuoles by recruiting Rab5 and Vps34. *J. Cell Biol.* **182**, 741–752 (2008).
43. Bakowski, M. A. *et al.* The phosphoinositide phosphatase SopB manipulates membrane surface charge and trafficking of the *Salmonella*-containing vacuole. *Cell Host Microbe* **7**, 453–462 (2010).
Demonstrates how the *S.* Typhimurium effector SopB exerts a global effect of recruiting host proteins by altering the surface charges of SCVs via its phosphatase activity.
44. Mason, D. *et al.* Alteration of epithelial structure and function associated with PtdIns(4,5)P_2 degradation by a bacterial phosphatase. *J. Gen. Physiol.* **129**, 267–283 (2007).
45. Wennerberg, K., Rossman, K. L. & Der, C. J. The Ras superfamily at a glance. *J. Cell Sci.* **118**, 843–846 (2005).
46. Stenmark, H. & Olkkonen, V. M. The Rab GTPase family. *Genome Biol.* **2**, REVIEWS3007 (2001).
47. Scheffzek, K. & Ahmadian, M. R. GTPase activating proteins: structural and functional insights 18 years after discovery. *Cell. Mol. Life Sci.* **62**, 3014–3038 (2005).
48. Pfeffer, S. & Aivazian, D. Targeting Rab GTPases to distinct membrane compartments. *Nature Rev. Mol. Cell Biol.* **5**, 886–896 (2004).
49. Vetter, I. R. & Wittinghofer, A. The guanine nucleotide-binding switch in three dimensions. *Science* **294**, 1299–1304 (2001).
50. Brumell, J. H. & Scidmore, M. A. Manipulation of Rab GTPase function by intracellular bacterial pathogens. *Microbiol. Mol. Biol. Rev.* **71**, 636–652 (2007).
51. Mukherjee, K., Parashuraman, S., Raje, M. & Mukhopadhyay, A. SopE acts as an Rab5-specific nucleotide exchange factor and recruits non-prenylated Rab5 on *Salmonella*-containing phagosomes to promote fusion with early endosomes. *J. Biol. Chem.* **276**, 23607–23615 (2001).
52. Bulgin, R. *et al.* Bacterial guanine nucleotide exchange factors SopE-like and WxxxE effectors. *Infect. Immun.* **78**, 1417–1425 (2010).
53. Murata, T. *et al.* The *Legionella pneumophila* effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nature Cell Biol.* **8**, 971–977 (2006).
Introduces the *L. pneumophila* Dot/Icm effector SidM, a RABGEF that is important for RAB1 recruitment to the LCV. This unique bacterial protein alters vesicle trafficking by targeting host RAB GTPases.
54. Muller, M. P. *et al.* The *Legionella* effector protein DrrA AMPylates the membrane traffic regulator Rab1b. *Science* **329**, 946–949 (2010).
55. Schoebel, S., Oesterlin, L. K., Blankenfeldt, W., Goody, R. S. & Itzen, A. RabGDI displacement by DrrA from *Legionella* is a consequence of its guanine nucleotide exchange activity. *Mol. Cell* **36**, 1060–1072 (2009).
56. Ingmundson, A., Delprato, A., Lambright, D. G. & Roy, C. R. *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. *Nature* **450**, 365–369 (2007).
Describes two *L. pneumophila* effectors, SidM and LepB, that act in recycling RAB1 on the LCV membrane. SidM has GEF activity, thereby aiding in RAB1 recruitment to the membrane; by contrast, LepB aids in the removal of RAB1 from the membrane by acting as a GAP.
57. Sturgill-Koszycki, S. & Swanson, M. S. *Legionella pneumophila* replication vacuoles mature into acidic, endocytic organelles. *J. Exp. Med.* **192**, 1261–1272 (2000).
58. Fu, Y. & Galan, J. E. A *Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. *Nature* **401**, 293–297 (1999).
59. Hardt, W. D., Chen, L. M., Schuebel, K. E., Bustelo, X. R. & Galan, J. E. S. typhimurium encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* **93**, 815–826 (1998).
60. Schlumberger, M. C. & Hardt, W. D. Triggered phagocytosis by *Salmonella*: bacterial molecular mimicry of RhoGTPase activation/deactivation. *Curr. Top. Microbiol. Immunol.* **291**, 29–42 (2005).
61. Kaniga, K., Uralil, J., Bliska, J. B. & Galan, J. E. A secreted protein tyrosine phosphatase with modular effector domains in the bacterial pathogen *Salmonella typhimurium*. *Mol. Microbiol.* **21**, 633–641 (1996).
62. Fu, Y. & Galan, J. E. The *Salmonella typhimurium* tyrosine phosphatase SptP is translocated into host cells and disrupts the actin cytoskeleton. *Mol. Microbiol.* **27**, 359–368 (1998).
63. Kubori, T. & Galan, J. E. Temporal regulation of salmonella virulence effector function by proteasome-dependent protein degradation. *Cell* **115**, 333–342 (2003).
Discusses the regulatory mechanism by which SopE and SptP exert their function in the host cell via a proteasome-dependent protein degradation pathway.
64. Riese, M. J. *et al.* Auto-ADP-ribosylation of *Pseudomonas aeruginosa* ExoS. *J. Biol. Chem.* **277**, 12082–12088 (2002).
65. Goehring, U. M., Schmidt, G., Pederson, K. J., Aktories, K. & Barbieri, J. T. The N-terminal domain of *Pseudomonas aeruginosa* exoenzyme S is a GTPase-activating protein for Rho GTPases. *J. Biol. Chem.* **274**, 36369–36372 (1999).
66. Von Pawel-Rammingen, U. *et al.* GAP activity of the *Yersinia* YopE cytotoxin specifically targets the Rho pathway: a mechanism for disruption of actin microfilament structure. *Mol. Microbiol.* **36**, 737–748 (2000).
67. Black, D. S. & Bliska, J. B. The RhoGAP activity of the *Yersinia pseudotuberculosis* cytotoxin YopE is required for antiphagocytic function and virulence. *Mol. Microbiol.* **37**, 515–527 (2000).
68. Casselli, T., Lynch, T., Southward, C. M., Jones, B. W. & DeVinney, R. *Vibrio parahaemolyticus* inhibition of Rho family GTPase activation requires a functional chromosome I type III secretion system. *Infect. Immun.* **76**, 2202–2211 (2008).
69. Ogawa, M., Handa, Y., Ashida, H., Suzuki, M. & Sasakawa, C. The versatility of *Shigella* effectors. *Nature Rev. Microbiol.* **6**, 11–16 (2008).
70. Alto, N. M. *et al.* Identification of a bacterial type III effector family with G protein mimicry functions. *Cell* **124**, 133–145 (2006).
71. Handa, Y. *et al. Shigella* IpgB1 promotes bacterial entry through the ELMO–Dock180 machinery. *Nature Cell Biol.* **9**, 121–128 (2007).
Shows that IpgB1 activates RAC1 by directly activating the ELMO–DOCK180 machinery, thereby inducing membrane ruffles and promoting bacterial entry.
72. Ohya, K., Handa, Y., Ogawa, M., Suzuki, M. & Sasakawa, C. IpgB1 is a novel *Shigella* effector protein involved in bacterial invasion of host cells. Its activity to promote membrane ruffling via Rac1 and Cdc42 activation. *J. Biol. Chem.* **280**, 24022–24034 (2005).
73. Huang, Z. *et al.* Structural insights into host GTPase isoform selection by a family of bacterial GEF mimics. *Nature Struct. Mol. Biol.* **16**, 853–860 (2009).
74. Klink, B. U. *et al.* Structure of *Shigella* IpgB2 in complex with human RhoA: implications for the mechanism of bacterial guanine nucleotide exchange factor mimicry. *J. Biol. Chem.* **285**, 17197–17208 (2010).
75. Tu, X., Nisan, I., Yona, C., Hanski, E. & Rosenshine, I. EspH, a new cytoskeleton-modulating effector of enterohaemorrhagic and enteropathogenic *Escherichia coli*. *Mol. Microbiol* **47**, 595–606 (2003).
76. Dong, N., Liu, L. & Shao, F. A bacterial effector targets host DH-PH domain RhoGEFs and antagonizes macrophage phagocytosis. *EMBO J.* **29**, 1363–1376 (2010).
77. Selyunin, A. S. *et al.* The assembly of a GTPase-kinase signalling complex by a bacterial catalytic scaffold. *Nature* **469**, 107–111 (2010).
Describes EspG, a unique bacterial effector that disrupts membrane trafficking and acts as a catalytic scaffold by inhibiting ARF-family GTPases and activating PAK proteins at membrane organelles.

78. Bruggemann, H. *et al.* Virulence strategies for infecting phagocytes deduced from the *in vivo* transcriptional program of *Legionella pneumophila*. *Cell. Microbiol.* **8**, 1228–1240 (2006).
79. Levine, B. & Kroemer, G. Autophagy in the pathogenesis of disease. *Cell* **132**, 27–42 (2008).
80. Gutierrez, M. G. *et al.* Autophagy is a defence mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* **119**, 753–766 (2004).
81. Rich, K. A., Burkett, C. & Webster, P. Cytoplasmic bacteria can be targets for autophagy. *Cell. Microbiol.* **5**, 455–468 (2003).
82. Dorn, B. R., Dunn, W. A., Jr & Progulske-Fox, A. Bacterial interactions with the autophagic pathway. *Cell. Microbiol.* **4**, 1–10 (2002).
83. Kirkegaard, K., Taylor, M. P. & Jackson, W. T. Cellular autophagy: surrender, avoidance and subversion by microorganisms. *Nature Rev. Microbiol.* **2**, 301–314 (2004).
Reviews the mechanism of autophagy, methods of monitoring the autophagic pathway, and the subversion of autophagy by bacteria and viruses.
84. Colombo, M. I. Pathogens and autophagy: subverting to survive. *Cell Death Differ.* **12** (Suppl. 2), 1481–1483 (2005).
85. Colombo, M. I. Autophagy: a pathogen driven process. *IUBMB Life* **59**, 238–242 (2007).
86. Dorn, B. R., Dunn, W. A. Jr & Progulske-Fox, A. Porphyromonas gingivalis traffics to autophagosomes in human coronary artery endothelial cells. *Infect. Immun.* **69**, 5698–5708 (2001).
87. Pizarro-Cerda, J. *et al. Brucella abortus* transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. *Infect. Immun.* **66**, 5711–5724 (1998).
88. Pizarro-Cerda, J., Moreno, E., Sanguedolce, V., Mege, J. L. & Gorvel, J. P. Virulent *Brucella abortus* prevents lysosome fusion and is distributed within autophagosome-like compartments. *Infect. Immun.* **66**, 2387–2392 (1998).
89. Comerci, D. J., Martinez-Lorenzo, M. J., Sieira, R., Gorvel, J. P. & Ugalde, R. A. Essential role of the VirB machinery in the maturation of the *Brucella abortus*-containing vacuole. *Cell. Microbiol.* **3**, 159–168 (2001).
90. Swanson, M. S. & Isberg, R. R. Association of *Legionella pneumophila* with the macrophage endoplasmic reticulum. *Infect. Immun.* **63**, 3609–3620 (1995).
91. Amer, A. O. & Swanson, M. S. Autophagy is an immediate macrophage response to *Legionella pneumophila*. *Cell. Microbiol.* **7**, 765–778 (2005).
92. Otto, G. P. *et al.* Macroautophagy is dispensable for intracellular replication of *Legionella pneumophila* in *Dictyostelium discoideum*. *Mol. Microbiol.* **51**, 63–72 (2004).
93. Tung, S. M. *et al.* Loss of *Dictyostelium* ATG9 results in a pleiotropic phenotype affecting growth, development, phagocytosis and clearance and replication of *Legionella pneumophila*. *Cell. Microbiol.* **12**, 765–780 (2010).
94. Beron, W., Gutierrez, M. G., Rabinovitch, M. & Colombo, M. I. *Coxiella burnetii* localizes in a Rab7-labeled compartment with autophagic characteristics. *Infect. Immun.* **70**, 5816–5821 (2002).
95. Gutierrez, M. G. *et al.* Autophagy induction favours the generation and maturation of the *Coxiella*-replicative vacuoles. *Cell. Microbiol.* **7**, 981–993 (2005).
96. Hernandez, L. D., Pypaert, M., Flavell, R. A. & Galan, J. E. A *Salmonella* protein causes macrophage cell death by inducing autophagy. *J. Cell Biol.* **163**, 1123–1131 (2003).
97. Beuzon, C. R. *et al. Salmonella* maintains the integrity of its intracellular vacuole through the action of SifA. *EMBO J.* **19**, 3235–3249 (2000).
98. Brawn, L. C., Hayward, R. D. & Koronakis, V. *Salmonella* SPI1 effector SipA persists after entry and cooperates with a SPI2 effector to regulate phagosome maturation and intracellular replication. *Cell Host Microbe* **1**, 63–75 (2007).
99. Schroeder, N. *et al.* The virulence protein SopD2 regulates membrane dynamics of *Salmonella*-containing vacuoles. *PLoS Pathog.* **6**, e1001002 (2010).
100. Murata-Kamiya, N., Kikuchi, K., Hayashi, T., Higashi, H. & Hatakeyama, M. *Helicobacter pylori* exploits host membrane phosphatidylserine for delivery, localization, and pathophysiological action of the CagA oncoprotein. *Cell Host Microbe* **7**, 399–411 (2010).
101. Hutagalung, A. H. & Novick, P. J. Role of Rab GTPases in membrane traffic and cell physiology. *Physiol. Rev.* **91**, 119–149 (2011).

Acknowledgements
We thank N. Alto and members of the Orth laboratory for their advice and discussion. K.O., H.H. and A.S. are supported by grants from the National Institute of Allergy and Infectious Diseases, US National Institutes of Health (R01-AI056404 and R01-AI087808) and the Welch Foundation (I-1561). A.S. is supported by the Howard Hughes Medical Institute Med to Grad Initiative. K.O. is a Burroughs Wellcome Investigator in Pathogenesis of Infectious Disease and a W. W. Caruth, Jr. Biomedical Scholar.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Kim Orth's homepage:
http://www4.utsouthwestern.edu/orthlab/