The Role of the Type III Secretion System in the Intracellular Lifestyle of Enteric Pathogens

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ABSTRACT Several pathogens have evolved to infect host cells from within, which requires subversion of many host intracellular processes. In the case of Gram-negative pathogenic bacteria, adaptation to an intracellular life cycle relies largely on the activity of type III secretion systems (T3SSs), an apparatus used to deliver effector proteins into the host cell, from where these effectors regulate important cellular functions such as vesicular trafficking, cytoskeleton reorganization, and the innate immune response. Each bacterium is equipped with a unique suite of these T3SS effectors, which aid in the development of an individual intracellular lifestyle for their respective pathogens. Some bacteria adapt to reside and propagate within a customized vacuole, while others establish a replicative niche in the host cytosol. In this article, we review the mechanisms by which T3SS effectors contribute to these different lifestyles. To illustrate the formation of a vacuolar and a cytosolic lifestyle, we discuss the intracellular habitats of the enteric pathogens *Salmonella enterica* serovar Typhimurium and *Shigella flexneri*, respectively. These represent well-characterized systems that function as informative models to contribute to our understanding of T3SS-dependent subversion of intracellular processes. Additionally, we present *Vibrio parahaemolyticus*, another enteric Gram-negative pathogen, as an emerging model for future studies of the cytosolic lifestyle.

INTRODUCTION

Many bacterial pathogens have evolved to infect host cells from the inside. In fact, some bacteria, such as *Rickettsia* spp. and *Coxiella* spp., are entirely reliant on host intracellular resources to propagate (1). The adaptation of bacteria to an intracellular lifecycle is thought to confer a means to avoid the harsh extracellular milieu (low pH, physical stress, host defenses), to gain access to a nutrient-rich environment, and to facilitate the spread of the pathogen to neighboring host tissues (2, 3).

The intracellular lifecycle of a bacterium initiates with its entry into a host cell. Cell entry can be a host-induced event, as in the case of bacterial uptake by phagocyte macrophages, or a bacteria-active process, as in the case of bacterial invasion of epithelial cells (4). Internalized bacteria are initially contained in a membrane-bound vacuole derived from the host cell plasma membrane (4). This vacuole is destined to traffic along the endocytic pathway, a route that defaults to vacuolar fusion with the lysosome, where vacuolar contents are degraded (i.e., bacterial killing) (5). To counteract this detrimental route, classic mechanisms used by pathogenic bacteria include avoidance of vacuole-lysosome fusion or conversion of the phagolysosomal environment into one permissive to bacterial survival (4, 5). In both instances, bacteria are referred to as vacuolar pathogens (4, 5).

Alternatively, some bacteria avoid lysosomal killing by disrupting the vacuole membrane and escaping into the host cytosol; these bacteria are termed cytosolic ([5](5)). The classic vacuolar/cytosolic distinction is not always clear because, under some circumstances, some vacuolar bacteria escape into the cytosol and some cytosolic bacteria are recompartmentalized into a vacuole ([5](5)).

Crafting an intracellular lifestyle requires bacterial subversion of the host cell's machinery. One virulence factor used to subvert cellular processes is the type III secretion system (T3SS), encoded by many pathogenic Gram-negative bacteria. The T3SS is a syringe-like secretory apparatus used by the bacteria to deliver a special set of proteins, effectors, into the host cytosol ([6](6)). The apparatus is composed of 20 to 30 proteins that are relatively well conserved among different pathogens ([6](6)). The apparatus assembly initiates with formation of a basal body containing two sets of rings spanning both the inner and outer bacterial membranes ([6](6)). The basal body projects a hollow, syringe-like conduit through which the effectors travel to the eukaryotic host cell ([6](6)). At the tip of the conduit lies a protein complex that upon sensing the host cell acts as a scaffold for the formation of a translocon pore on the host cell membrane ([6](6)). The effectors, delivered through the pore, are often mimics of eukaryotic proteins; coevolution with their hosts led bacteria to usurp host protein functionalities, which were then subverted to facilitate infection ([7](7), [8](8)). Cellular processes commonly disrupted by T3SS effectors include the innate immune response, the cytoskeleton machinery, and cargo trafficking ([7](7), [8](8)).

It is expected that pathogens will distinctively employ their T3SSs to support growth inside a vacuole versus growth in the host cytosol. In this article we discuss how T3SSs promote the intracellular lifestyle of *Salmonella enterica* serovar Typhimurium and *Shigella flexneri*. Both bacteria are well-characterized enteric pathogens; the former is the causative agent of salmonellosis, one the most common foodborne illnesses, and the latter is the major causal agent of bacillary dysentery ([8](8), [9](9)). *S.* Typhimurium is primarily a vacuolar pathogen, while *S. flexneri* colonizes the host cytosol ([8](8), [9](9)). Therefore, a parallel comparison of these two pathogens' intracellular lifestyles provides a comprehensive overview of the mechanisms used by T3SSs to subvert cellular functions. Additionally, we discuss *Vibrio parahaemolyticus*, a major cause of seafoodborne enteritis ([10](10)). Recently, it was revealed that this bacterium adopts a T3SS-dependent intracellular lifecycle, positioning *V. parahaemolyticus* as a model for future discoveries of T3SS-mediated intracellular subversion ([11](11), [12](12)).

S. TYPHIMURIUM AND LIFE IN THE *SALMONELLA*-CONTAINING VACUOLE: ROLE OF THE T3SS IN VACUOLE BIOGENESIS AND MAINTENANCE

S. Typhimurium invades intestinal epithelial cells through the activity of its first T3SS, *Salmonella* pathogenicity island 1 (SPI-1) ([9](9)). Following cell entry, the bacterium is contained within a unique membranous compartment, the *Salmonella*-containing vacuole (SCV), which transiently acquires early endosomal features ([9](9)). Later, the SCV matures through selective attainment of late endosomal and lysosomal content but does not become bactericidal ([9](9)). Activation of the second T3SS, SPI-2, occurs several hours after invasion, and from that moment on, SPI-2 effectors work to adapt the SCV to a replicative niche ([7](7)).

Therefore, SPI-1 effectors are not only critical during bacterial cell invasion, but also regulate the early steps of SCV biogenesis. The SPI-1 effectors SopE and SopE2 are homologs to each other and mimics of host guanidine exchange factors (GEF), triggering the release of GDP to facilitate GTP-binding and activation of Cdc42 and Rac1 ([13](13)–[15](15)). SopE is sufficient to promote plasma membrane ruffling and *S.* Typhimurium invasion ([15](15)). While most SopE/E2 becomes degraded shortly after bacterial invasion ([16](16)), a small fraction of these effectors remain active for several hours postinvasion ([17](17)). These active pools of SopE/E2 localize to the membrane of the nascent SCV ([17](17)), where they activate the small GTPase Rab5 through their GEF activity. Rab5 promotes homotypic fusion between early-endosomal compartments and fusion events between the SCV and early endosomes ([18](18)).

The SPI-1 effector SopB (also known as SigD) is a phosphatidylinositol phosphatase that hydrolyzes a wide range of phosphoinositide substrates *in vitro* ([19](19)) with specific phosphatidylinositol 4,5-biphosphate phosphatase [$PI(4,5)P_2$] activity *in vivo* ([20](20)). Although not essential for cell invasion, SopB plays an important role in nascent SCV formation ([21](21)–[23](23)). Depletion of $PI(4,5)P_2$ by SopB promotes fusion between the SCV and vesicles containing Rab5 ([23](23)). One of the Rab5 effectors that is associated with the SCV is Vps34, a phosphatidylinositol-3 (PI-3) kinase ([21](21), [23](23)). Vps34 phosphorylation of local pools of phosphatidylinositol (PI) forms phosphatidylinositol 3-phosphate [$PI(3)P$] that recruits the early endosomal component early-endosome antigen 1 (EEA1) to the SCV membrane ([21](21), [23](23)). Therefore, the early biogenesis of the SCV is dependent on the activity of SopB, which promotes the acquisition of Rab5, PI(3)P, and EEA1.

The maturation of the SCV leads to the progressive loss of these early endosomal markers and acquisition

of late endosomal content, such as lysosome-associated membrane protein 1 (Lamp-1) (24). Lamp-1 recruitment to the SCV is dependent on Rab7, which accumulates on the SCV early during infection (maximal level of association at 40 min postinfection) (24). Rab7 is a key regulator of late endosome trafficking to lysosomal compartments (25). During this process, Rab7 binds one of its host signaling partners, Rab-7 interacting lysosomal protein (RILP), which engages the late endosomes to the microtubule motor complex dynein-dynactin (25). This enables movement of late endosomes along microtubules toward the lysosomes located at the minus end of the microtubule-organizing center (25). Therefore, by recruiting Rab7 to the SCV, *S.* Typhimurium hijacks the endocytic pathway to promote the translocation of the SCV to the host cell perinuclear region (26, 27).

While Rab7-dependent juxtanuclear positioning of the SCV is crucial for SCV development, *Salmonella* must impede Rab7-mediated transport of the SCV to lysosomal (bactericidal) compartments. One mechanism to avoid lysosomal degradation of the SCV is to block the activity of Rab7. The SPI-2 effector SopD2 localizes to the SCV via its N-terminal domain, which also binds to Rab7 (28). SopD2 association with Rab7 precludes the interaction of the small GTPase with RILP, thereby preventing delivery of the SCV to the lysosome (28).

SopD2 further limits the interaction of the SCV with lysosomal compartments through its concerted activity with GtgE, another SPI-1 effector. GtgE is a cysteine protease that specifically cleaves the switch I region of the highly homologous Rabs 28, 29, and 32 when in their GDP-bound form, thereby disrupting interactions with downstream signaling partners (29–32). Rab32 is involved in the biogenesis of lysosome-related organelles, an intracellular membrane-bound compartment that shares many features with endosomes and lysosomes, such as Lamp-1 recruitment and acidic luminal pH (33). Because the SCV is similar to lysosome-related organelles, it can accumulate Rab32. In fact, the SCV of *S. enterica* serovar Typhi (the causal agent of typhoid fever), which lacks GtgE, accumulates Rab32, resulting in lower intracellular replicative rates than its GtgE-carrying counterpart, *S.* Typhimurium (29). Interestingly, while GtgE reduces Rab32 availability, the SCV of the *S.* Typhimurium *gtgE⁻* mutant still maintains poor association with Rab32, indicating that an additional effector(s) is involved in precluding Rab32 from this vacuole (34). The activity of SopD2 provides a striking example of cooperation between effectors. SopD2, unlike GtgE, does not affect the cellular levels of Rab32; instead, it inactivates Rab32 by accelerating hydrolysis of Rab32-GTP through its C-terminally encoded guanine-activating protein domain (34). Thereby, SopD2 enriches for Rab32-GDP, an inactive GTPase that does not interact with the SCV and the preferred form of Rab32 as the substrate for GtgE (34).

In addition to its role in early SCV biogenesis, SopB also limits SCV acquisition of late-endosomal and lysosomal content as a mechanism to avoid bacterial degradation. SopB-mediated formation of PI(3)P on the SCV allows for recruitment of sorting nexin-1, which is a component of the retromer complex involved in recycling of endosomal proteins to the trans-Golgi network (35). Sorting nexin-1 prevents accumulation of the late endosomal protein cation-independent mannose 6-phosphate receptor (MPR) on the SCV (35). Importantly, SopB-mediated hydrolysis of PI(4,5)P$_2$ at the plasma membrane generates SCVs devoid of this phosphoinositide, which is a negatively charged lipid that contributes to the net negative surface charge of the inner leaflet of the plasma membrane (36). Rab35, which promotes phagosomal-lysosomal fusion, localizes to the plasma membrane through these electrostatic interactions (36). As a result, in the absence of PI(4,5)P$_2$ the SCV membrane cannot be targeted by Rab35, and SCV-lysosome fusion is prevented (36).

While the initial nuclear apposition of the SCV is not SPI-2 dependent, the retention of the vacuole in this position is dependent on three SPI-2 effectors: SseG, SseF, and SifA (26, 37, 38). SseG and SseF share about 35% amino acid identity, bind to each other, and both interact with the Golgi network-associated protein 60/Golgi protein acyl coenzyme A binding domain-containing 3 (GCP60/ACBD3) (39, 40). The SseG, SseF, and GCP60/ACBD3 complex tethers the SCV to the Golgi network, sustaining the SCV in its perinuclear position (40). In fact, SseG-mediated interaction with the Golgi network was shown to restrict SCV motility (26).

An additional strategy to maintain the SCV in its juxtanuclear position involves antagonizing the activity of kinesin-1, a microtubule motor protein that mediates cargo trafficking toward the cell periphery, plus-end of the microtubule-organizing center (41). PipB2, an SPI-2 effector, localizes to the SCV and directly binds kinesin light chain 1, thereby recruiting kinesin-1 to the SCV (42, 43). The accumulation of kinesin-1 directs movement of the SCV to the cell periphery and scattering of these vacuoles (44). To counteract anterograde SCV redistribution, the bacterium employs another SPI-2 effector, SifA, that interacts with the host protein SKIP (SifA and kinesin-interacting protein) and binds kinesin-1, inhibiting the centrifugal movement of the SCV (44). Therefore, *S.* Typhimurium balances the antagonizing activities of

PipB2 and SifA in such a way that the inhibitory activity of SifA predominates over the activating role of PipB2 for kinesin-1 to maintain the SCV juxtanuclear position ([43](#)).

SipA is an SPI-1 effector known for its contribution during host cell invasion. This effector promotes actin polymerization and stability of actin filaments, thereby enhancing the local concentration of F-actin necessary to support membrane ruffle entry structures ([45](#)–[47](#)). Like the SPI-1 effectors discussed above, SipA remains active after *S.* Typhimurium entry into host cells ([48](#)). Following apposition to the nucleus, the SCV is stabilized by an F-actin meshwork that is less evident during *sipA*⁻ mutant infections, consistent with SipA targeting of actin ([48](#)). The F-actin stabilization of the SCV in a SipA-dependent manner is important to maintain the localization of SifA on the SCV ([48](#)). In the absence of SipA, SifA exhibits poor localization to the SCV, which results in PipB2/kinesin-mediated scattering of the SCV to the cell periphery ([48](#)). Therefore, SipA cooperates to localize SifA to the SCV, thereby maintaining the nuclear positioning of the SCV.

Following apposition to the nucleus, the SCV develops tubular extensions known as *Salmonella*-induced filaments (Sifs) ([49](#)). Sif formation coincides with the onset of *S.* Typhimurium intracellular replication ([50](#)), and disruption of Sif formation correlates with attenuated virulence *in vivo* ([51](#)). Sifs elongate along microtubules in a centrifugal fashion, which implicates PipB2/kinesin-1 in this process ([52](#), [53](#)). In fact, SCVs formed with infection of a *pipB2*⁻ *S.* Typhimurium mutant result in shorter filaments compared to wild-type bacteria ([42](#)). SifA is the principal effector involved in Sif formation. SifA binds active Rab7 on Sif membranes and impedes the interaction of the small GTPase with its effector RILP ([54](#)). RILP is thereby excluded from Sifs and cannot recruit dynein, which precludes retrograde extension of the filament ([54](#)). SifA also binds the N-terminal domain RUN (RPIP8, UNC-14, and NESCA) of SKIP ([53](#)). SifA and SKIP then interact with kinesin-1 and trigger the fission of SCV-derived PipB2/kinesin-1 vesicles, whose anterograde movement contributes to Sif growth ([53](#)).

The SPI-1 effector SptP further promotes Sif formation. The N terminus of SptP contains a guanine-activating protein domain that inactivates Rac1 and Cdc42, enabling the actin cytoskeleton to recover its normal appearance after *S.* Typhimurium invasion ([55](#)). SptP localizes to the SCV and persists there for many hours after bacterial invasion ([56](#)). The postinvasion role of SptP relies on its C-terminally encoded phosphatase domain ([56](#), [57](#)). SptP directly binds to and activates, via dephospho-rylation, the valosin-containing protein, a member of the AAA+ (ATPase associated with diverse cellular activities) family of ATPases. Dephosphorylated valosin-containing protein participates in vesicle fusion by binding to the t-SNARE (*N*-ethylmaleimide-sensitive-factor attachment protein receptor) syntaxin 5. Thereby, SptP promotes membrane fusion events that contribute to Sif formation and biogenesis of the *S.* Typhimurium intracellular replicative niche ([56](#)).

SifA is also involved in both inhibiting and promoting trafficking of lysosomal content to the SCV. Newly synthesized hydrolytic enzymes in the trans-Golgi network are transported to endosomes through cation-dependent and cation-independent MPRs ([58](#)). Endosomal maturation promotes activation of these hydrolases, which are then transported to lysosomes ([58](#)). One pathway that mediates recycling of MPRs from endosomes back to the trans-Golgi network involves the SNARE syntaxin 10 and its upstream effector Rab9 ([58](#)). SifA and SKIP sequester Rab9, thereby subverting the Rab9-dependent recycling of MPR, which compromises lysosomal function ([58](#)).

The fusion of lysosomes with membrane-bound compartments requires Rab7, as previously discussed, as well as the small GTPase Arl8b and the tethering factor HOPS (homotypic fusion and protein sorting) complex ([59](#)). HOPS is a hexameric complex whose subunit Vps (vacuole protein sorting) 41 is targeted to lysosomes via Arl8b. Another HOPS subunit, Vps39, interacts with SKIP ([59](#)). During *S.* Typhimurium infection, Arl8b localization to SCV allows recruitment of the HOPS complex ([59](#)). SKIP, localized to SCV and Sifs through binding to SifA, is also involved in recruitment of the HOPS complex ([59](#)). Tethering of the HOPS complex to the SCV enables fusion of late endosomal and lysosomal content with the SCV, which is important for Sif formation and nutrient access that support bacterial intravacuolar replication ([59](#)).

In addition to SifA, the regulation of the SCV and Sif membrane dynamics appears to involve another SPI-2 effector, SseJ. During infection with *S.* Typhimurium *sifA*⁻ mutants, the SCV is destabilized, causing the bacterium to escape from the vacuole into the host cytosol to experience either robust replication in epithelial cells or death in macrophages ([60](#), [61](#)). However, during infection with the *S.* Typhimurium *sifA*⁻ *sseJ*⁻ double mutant, the SCV remains intact, ascribing a role for SseJ in vacuolar membrane loss ([61](#)). SseJ belongs to the GDSL motif-containing family of lipases and shares 29% amino acid identity with the glycerophospholipid-cholesterol acyltransferase (GCAT) enzyme members of this family. GCAT enzymes catalyze the transfer of fatty acid acyl

groups from phospholipids to cholesterol to form cholesterol esters ([62](62)). SseJ exhibits deacylase, phospholipase A, and acyltransferase activities ([62](62)–[66](66)). Importantly, the enzymatic activity of SseJ is potentiated upon its binding to the active, GTP-bound form of RhoA ([65](65), [66](66)).

The esterification of cholesterol by SseJ results in the accumulation of cholesterol esters in the form of lipid droplets in infected cells, with the concurrent depletion of cholesterol from the plasma membrane and perinuclear region ([63](63)). In the absence of SseJ, cholesterol is found on the SCV and Sif membranes, whereas an excess of SseJ inhibits Sif formation. Therefore, SseJ appears to modulate membrane dynamics by regulating cholesterol levels ([61](61), [63](63)). The SseJ-mediated loss of SCV membrane integrity in the absence of SifA and inhibited Sif formation upon SseJ overexpression suggest an antagonist relationship between SifA and SseJ ([61](61)–[63](63)). Interestingly, SifA and SseJ were found to form a protein complex with RhoA, resulting in the formation of tubular extensions reminiscent of Sif filaments. These observations support a cooperative interaction between SseJ and SifA to fine-tune the membrane composition of SCV and Sifs ([67](67)).

One other T3SS effector involved in SCV and Sif membrane dynamics is SteA. This effector is one of a few *S.* Typhimurium T3SS effectors translocated by both SPI-1 and SPI-2 ([68](68), [69](69)). Bacterially translocated SteA localizes to the SCV and Sif membranes in a phosphatidylinositol 4-phosphate [PI(4)P]-dependent manner ([70](70)). The molecular target(s) of SteA remain uncharacterized, but deletion of this effector results in compact SCVs that contain several bacteria (as opposed to an SCV containing a single wild-type *S.* Typhimurium bacterium) and display a decreased number of Sifs ([71](71)). This mutant phenotype can be counteracted with pharmacological inhibition of dynein and kinesin, implicating SteA in the regulation of the activity of these microtubule protein motors ([71](71)).

Several hours (6 to 7 h) after bacterial invasion of epithelial cells, the SCV becomes surrounded by an F-actin meshwork ([72](72)). As previously discussed, the SPI-1 effector SipA participates in this process, and SipA-mediated actin accumulation around the SCV maintains Sif localization to the SCV as well as SCV perinuclear positioning ([48](48)). SPI-2 effectors also regulate actin assembly near the SCV, and this appears to contribute to maintenance of the vacuole integrity ([72](72)). The SPI-2 effector SteC is sufficient to induce F-actin accumulation around the SCV ([73](73)). At its C terminus, SteC contains a kinase domain ([73](73)) that mediates phosphorylation of MEK1, resulting in a conformational change that induces MEK-autophosphorylation and activation ([74](74)).

MEK1 then stimulates a signaling cascade that includes extracellular signal-regulated kinase, myosin light chain kinase, and myosin II. The latter is responsible for the bundling of actin filaments as is observed upon ectopic expression of SteC ([74](74)).

The accumulation of F-actin around the SCV results from the *de novo* actin assembly, i.e., polymerization of actin monomers (G-actin) instead of local recruitment of preexisting filaments ([72](72)). As with many other cellular events regulated by *S.* Typhimurium, the formation of an F-actin meshwork surrounding the SCV is also the product of a bacterial fine-tuning of effectors with antagonizing activities. The SPI-2 effector SpvB, which inhibits actin polymerization by ADP-ribosylating G-actin, offsets the SteC-induced F-actin meshwork ([75](75)–[77](77)).

Altogether, these SPI effectors commandeer host vesicular trafficking and the cytoskeleton to establish a vacuolar replicative niche for *S.* Typhimurium while avoiding lysosomal degradation ([Fig. 1](Fig. 1) and Table 1).

S. FLEXNERI AND LIFE IN THE HOST CYTOSOL: ROLE FOR T3SS IN VACUOLE ESCAPE

The development of shigellosis starts with *S. flexneri* penetration of the intestinal epithelial barrier through the M cells that overlay lymphoid nodules ([3](3), [78](78)). Once reaching the underlying lymphoid tissue, *Shigella* is phagocytosed by resident macrophages, and shortly after, ruptures the phagosome to escape into the cytoplasm, where it initiates replication ([3](3), [78](78)). *Shigella*-induced death of the macrophages releases cytoplasmic bacteria that subsequently invade the neighboring enterocytes through their basolateral surface ([3](3), [78](78)). Following enterocyte invasion, *S. flexneri* lyses its vacuole to replicate within and move across the host cytosol ([3](3), [78](78)). The encounter of a motile bacterium with the cell plasma membrane generates a protrusion that forces the bacterium into adjacent enterocytes ([3](3), [78](78)). Movement across two cell plasma membranes (from primary and secondary invaded host cells) causes bacterial entry into the adjacent cell through a double-membrane vacuole that is also lysed by *S. flexneri* to then initiate another round of infection ([3](3), [78](78)).

Therefore, the ability of *Shigella* to escape from a vacuole, be it the macrophage phagosome or the single- or double-membrane enterocyte vacuole, is paramount for this bacterium's virulence. Vacuole escape for *Shigella* is a T3SS-dependent mechanism ([3](3)). One T3SS effector involved in this process is IpgD, a $PI(4,5)P_2$ 4-phosphatase ([79](79)). Hydrolysis of $PI(4,5)P_2$ by this effector disrupts the
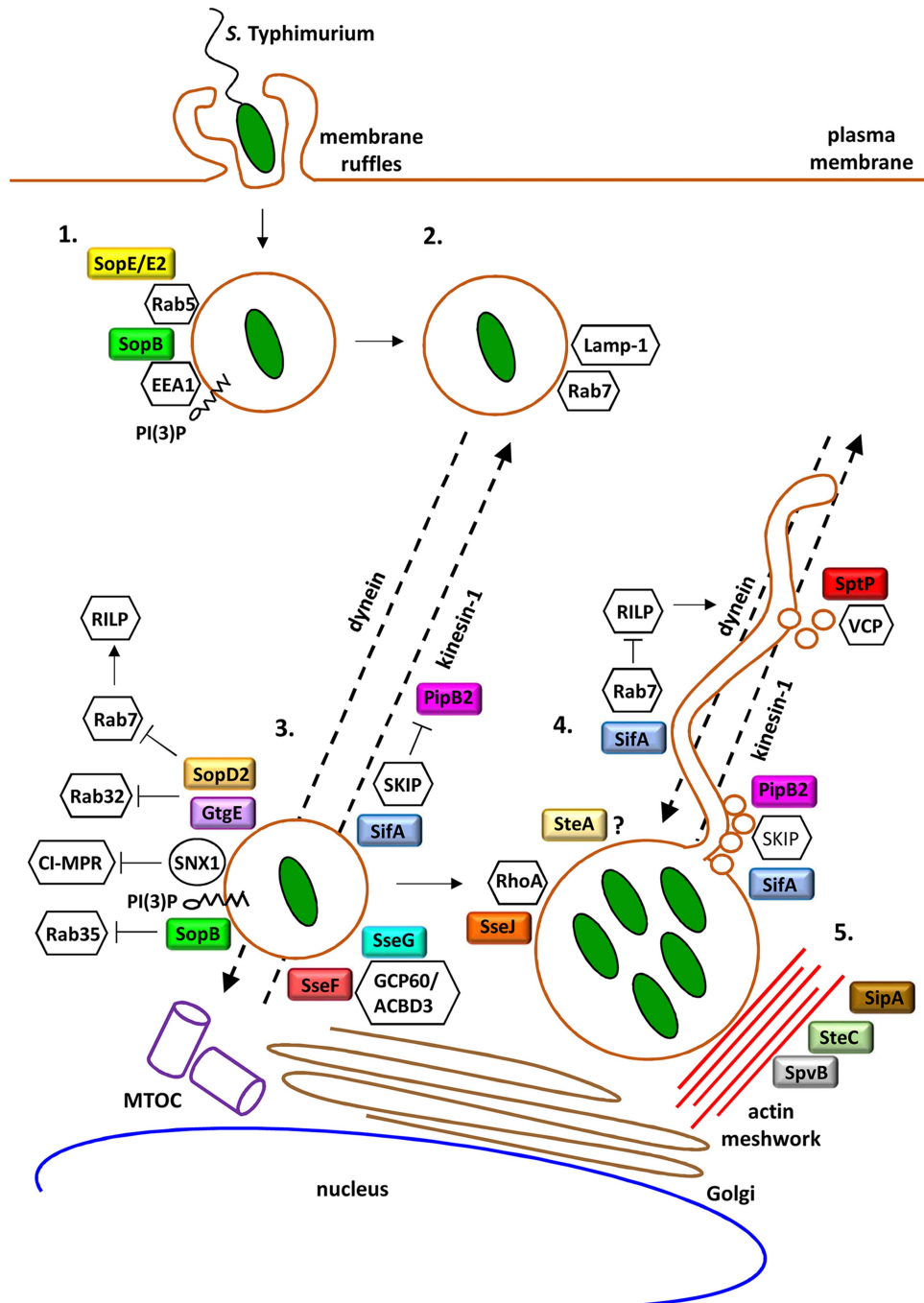
FIGURE 1 Schematic of the contribution of T3SS effectors to the intracellular lifecycle of *S.* Typhimurium **(1)**. Following invasion of epithelial cells, *S.* Typhimurium employs the effectors SopE/E2 and SopB to transiently recruit early endosomal markers to the *Salmonella*-containing vacuole (SCV) **(2)**. SCV maturation and acquisition of Rab7 lead to dynein-mediated translocation of this vacuole to the host cell perinuclear region **(3)**. Development of the SCV into a bactericidal compartment is precluded by the action of SopB, GtgE, and SopD2, which collectively, inhibit the endosomal-lysosomal fusion activities of Rab35, Rab32, and Rab7. SseF and SseG, in complex with GCP60/ACBD3, maintain the SCV juxtanuclear position. Additionally, SifA, through its eukaryotic effector SKIP, inhibits PipB2/kinesin-1-mediated centrifugal movement of the SCV **(4)**. Next, the SCV develops Sifs, which coincides with the onset of *S.* Typhimurium intravacuolar replication. Sif formation is the product of the concerted action of SifA, PipB2, and SseJ. SifA and PipB2 coordinate the centrifugal extension of the Sifs, while SifA and SseJ regulate SCV and Sif membrane dynamics that support Sif growth. Filament growth is further promoted by SptP **(5)**. The SCV is surrounded by an actin meshwork which contributes to maintaining SCV integrity. The effectors SipA, SpvB, and SteC modulate actin dynamics in the surroundings of the SCV.

TABLE 1 SPI-1 and SPI-2 effectors that contribute to the intracellular lifecycle of *S.* Typhimurium

Effector	Secretion system	Cellular target	Biochemical activity	Biological function	References
SopE/E2	SPI-1	Cdc42 and Rac1	GEF	SCV formation	[13]–[18]
SopB	SPI-1	PI(4,5)P$_2$	Phosphatidylinositol phosphatase	SCV formation, maturation, and avoidance of lysosomal degradation	[19]–[23], [35], [36]
GtgE	SPI-1	Rab32	Cysteine protease	SCV avoidance of lysosomal degradation	[29]–[34]
SopD2	SPI-2	Rab32, Rab7	GAP	SCV avoidance of lysosomal degradation	[28], [34]
SseG	SPI-2	GCP60/ACBD3	Unknown	SCV perinuclear positioning	[26], [37], [39], [40]
SseF	SPI-2	GCP60/ACBD3	Unknown	SCV perinuclear positioning	[37]–[40]
SifA	SPI-2	SKIP	Unknown	SCV perinuclear positioning, Sif formation, SCV integrity	[43], [44], [48], [49], [52]–[54], [58], [59]
SipA	SPI-1	Actin	Actin polymerization,	SCV perinuclear positioning	[45]–[48]
PipB2	SPI-2	Kinesin-1	Unknown	Sif formation	[42]–[44], [48], [52], [53]
SptP	SPI-1	Valosin-containing protein	Phosphatase	Sif formation	[55]–[57]
SseJ	SPI-2	Cholesterol, RhoA	Glycerophospholipid: cholesterol acyltransferase	SCV and Sif membrane dynamics	[60]–[67]
SteA	SPI-1, SPI-2	Unknown	Unknown	SCV and Sif membrane dynamics	[68]–[71]
SteC	SPI-2	MEK1	Kinase	SCV-surrounding actin meshwork	[73], [74]
SpvB	SPI-2	G-actin	ADP-ribosylating protein	SCV-surrounding actin meshwork	[75]–[77]

contact between cortical actin and the plasma membrane, contributing to formation of plasma membrane ruffles at bacterial entry sites ([79]). Collapse of membrane ruffles and fusion with the plasma membrane leads to engulfment of the bacterium in a process similar to macropinocytosis ([80]). Interestingly, IpgD enhances invasion efficiency but is not required for this process ([81]). Internalized *Shigella* is briefly contained within a tight, uniform vacuole (*S. flexneri*-containing vacuole, *Sf*CV) that ruptures 10 min after invasion ([80]). Initially, the *Sf*CV is surrounded by macropinosomes formed as a result of the IpgD-ruffling activity ([80]). The small GTPase Rab11, known to primarily associate with recycling endosomes, is directly recruited to the *Sf*CV-surrounding macropinosomes in an IpgD-dependent manner ([80], [82]). Once the Rab11-macropinosomes come in contact with *Sf*CV, the bacterial vacuole ruptures by a not yet defined mechanism ([80], [82]). In the absence of IpgD, the availability of macropinosomes is diminished, and a delay in bacterial vacuole escape is observed ([82]). Additionally, the *Sf*CV of *Shigella ipgD⁻* mutants is surrounded by an actin meshwork (actin cage) that obstructs *Shigella*'s escape ([82]).

The overall delay effect indicates that, in addition to IpgD, other T3SS components may be playing a role in vacuole rupture. IpaB and IpaC are components of the T3SS apparatus, specifically, translocon proteins that insert into host membranes through their hydrophobic regions to form membrane pores ([83], [84]). The translocon-pore activity of each of these two proteins contributes to destabilizing the membrane of phagosomes, in macrophages, as well as the entry and protrusion vacuole

membranes of infected epithelial cells, allowing *S. flexneri* to escape into the host cytosol ([85]–[88]). An elegant study demonstrated that the reconstitution of *Shigella*'s T3SS apparatus into a nonpathogenic *Escherichia coli* strain was sufficient to promote bacterial escape from a vacuole ([89]).

Following escape into the host cytosol, *S. flexneri* employs the secreted protein IcsA (also known as VirG) to spread both across and between epithelial cells ([90]). IcsA is a type V secreted autotransporter that uses the Sec secretion pathway to translocate across the bacterial inner membrane ([91]). Despite not being a T3SS effector, IcsA plays a seminal role to the intracellular lifestyle of *S. flexneri* and, therefore, merits discussion here. IcsA is delivered to the surface of the bacterium's old pole, and this polarized distribution is sustained by outer membrane properties such as fluidity and by IcsP-mediated proteolysis of nonpolarized IcsA ([92], [93]). The C-terminal, transporter domain of IcsA inserts into the bacterial outer membrane, while the N-terminal, passenger domain is exposed on the bacterial surface ([94]). The passenger domain specifically recruits and activates neural Wiskott-Aldrich syndrome protein (N-WASP) ([94]–[96]). N-WASP possesses several domains: an N-terminal WASP homology 1, a central GTPase-binding (GDB), and a C-terminal verprolin homology/cofilin/acidic (VCA) domain. The inactive conformation of N-WASP is established through the auto-inhibitory intramolecular interaction between the GDB and VCA domains; upon association of the Rho-GTPase Cdc42 with the GDB domain, the VCA domain is released, leading to the activation of

N-WASP ([97]). The IcsA passenger domain directly binds both the WASP homology 1 and the GBD domain, exposing the VCA domain that subsequently binds G-actin and activates the actin filament nucleator Arp2/3 complex ([94]). The IcsA-mediated unidirectional actin polymerization leads to the polarized formation of an actin comet-like tail that propels the bacterium forward during intra- and intercellular motility ([98]).

Actin-dependent movement of *S. flexneri* enables the bacterium to protrude and enter the neighboring cell via a double-membrane vacuole (protrusion *Sf*CV) ([3], [78]). The T3SS effector IcsB facilitates escape from this vacuole ([99]–[101]), albeit with no role in bacterial escape from the single-membrane (entry) vacuole. IcsB binds cholesterol through its cholesterol-binding domain ([102]). Because there is a noted difference in plasma membrane leaflet orientation in single- and double-membrane vacuoles and membrane cholesterol content, this could account for IcsB's specific activity ([101]).

The protrusion *Sf*CV is targeted for autophagy through the recruitment of the autophagosome marker light chain 3 (LC3) ([101]). Previous works attributed a role to IcsB in autophagy evasion because higher numbers of LC3-positive vacuoles were present during infections with *icsB*$^{-/-}$ mutants compared to the parental strain ([100], [102], [103]). However, a recent study demonstrated that vacuoles containing either wild-type or *icsB*$^{-/-}$ bacteria equally recruit LC3, with the failure of vacuole escape in the absence of IcsB being causal for LC3 enrichment ([101]).

LC3 recruitment to the protrusion *Sf*CV can be modulated by the T3SS effector VirA ([100], [104]). VirA is a guanine-activating protein that preferentially targets the GTPase Rab1 ([104]). In addition to its well-established role in regulating endoplasmic reticulum-Golgi and intra-Golgi trafficking, Rab1 is also involved in autophagosome formation ([105]). Therefore, it has been proposed that VirA hydrolyzes Rab1 as a mechanism to control antibacterial autophagy ([104]).

The entry into a host cell, the escape from the vacuoles, and the movement of *Shigella* from one cell to another are mediated by a small number of effectors that use the host cell resources to facilitate invasion, replication, and virulence ([Fig. 2] and [Table 2]).

V. PARAHAEMOLYTICUS: AN INTRACELLULAR BACTERIUM REVEALING NEW MECHANISMS FOR SURVIVAL AND REPLICATION

The diversity of T3SS-dependent mechanisms of intracellular subversion devised by *S.* Typhimurium and *S.* *flexneri* underscores the uniqueness of each bacterium's intracellular lifestyle and advocates for the investigation of new bacterial models as a way to uncover yet unknown mechanisms. The marine bacterium *V. parahaemolyticus* was first identified in 1950 as the causative agent of a diarrheal outbreak in Japan ([106]). The sequencing of the *V. parahaemolyticus* genome revealed the presence of two T3SSs: the first apparatus, T3SS1, was ancestrally acquired and is present in both environmental and clinical strains, and the second apparatus, T3SS2, was recently acquired (via horizontal transfer) and is present exclusively in clinical strains ([107]). T3SS2 is the virulence factor that governs acute gastroenteritis, the bacterium's principal manifestation in humans ([108]).

Since its discovery, this bacterium has been regarded as an exclusive extracellular bacterium, i.e., one that resides and propagates entirely outside of a host cell during infection. In fact, it was demonstrated that, *in vitro*, the potent cytotoxicity of the first T3SS masks the activity of the T3SS2 (albeit with no significant role for enterotoxicity; the T3SS1 can be activated upon culturing of the bacterium in tissue culture growth media) ([109], [110]). The T3SS1 effectors work in a temporal manner to orchestrate the death of the host cell within about 3 hours: first, VopQ inhibits autophagic flux by disrupting the host lysosomal V-ATPase; second, VPA0450 induces plasma membrane blebbing by hydrolyzing PI(4,5)P$_2$; third, VopS contains a Fic domain that AMPylates Rho GTPases, resulting in cell rounding ([111]). These events, and possibly the activity of one other uncharacterized effector, VopR ([112]), contribute to the final lysis of the host cell.

The use of a bacterial strain lacking both the hemolysins and the T3SS1 provided insight into the pathogenesis of the T3SS2 and revealed an intracellular lifecycle for *V. parahaemolyticus*. The T3SS2 effector VopC is a homolog of cytotoxic necrotizing factors that catalyzes the deamidation of Rho GTPases, specifically, Cdc42 and Rac1 ([11]). As a result, the deamidated Cdc42 and Rac1 adopt a constitutively active conformation resulting in dramatic rearrangements of the actin cytoskeleton ([11]). At sites of bacterial contact with the host epithelial cells, active Cdc42 and Rac1 reorganize the actin into membrane ruffles that promote the engulfment of *V. parahaemolyticus*, enabling bacterial invasion in nonphagocytic cells ([Fig. 3]) ([11], [12]). Upon uncovering VopC's activity, it became clear that *V. parahaemolyticus* is a facultative intracellular bacterium, i.e., one that resides and propagates both outside and inside of its host cell.

Following VopC-mediated invasion of epithelial cells, *V. parahaemolyticus* is enclosed within a vacuole that interacts with the endocytic pathway. The vacuole tran-
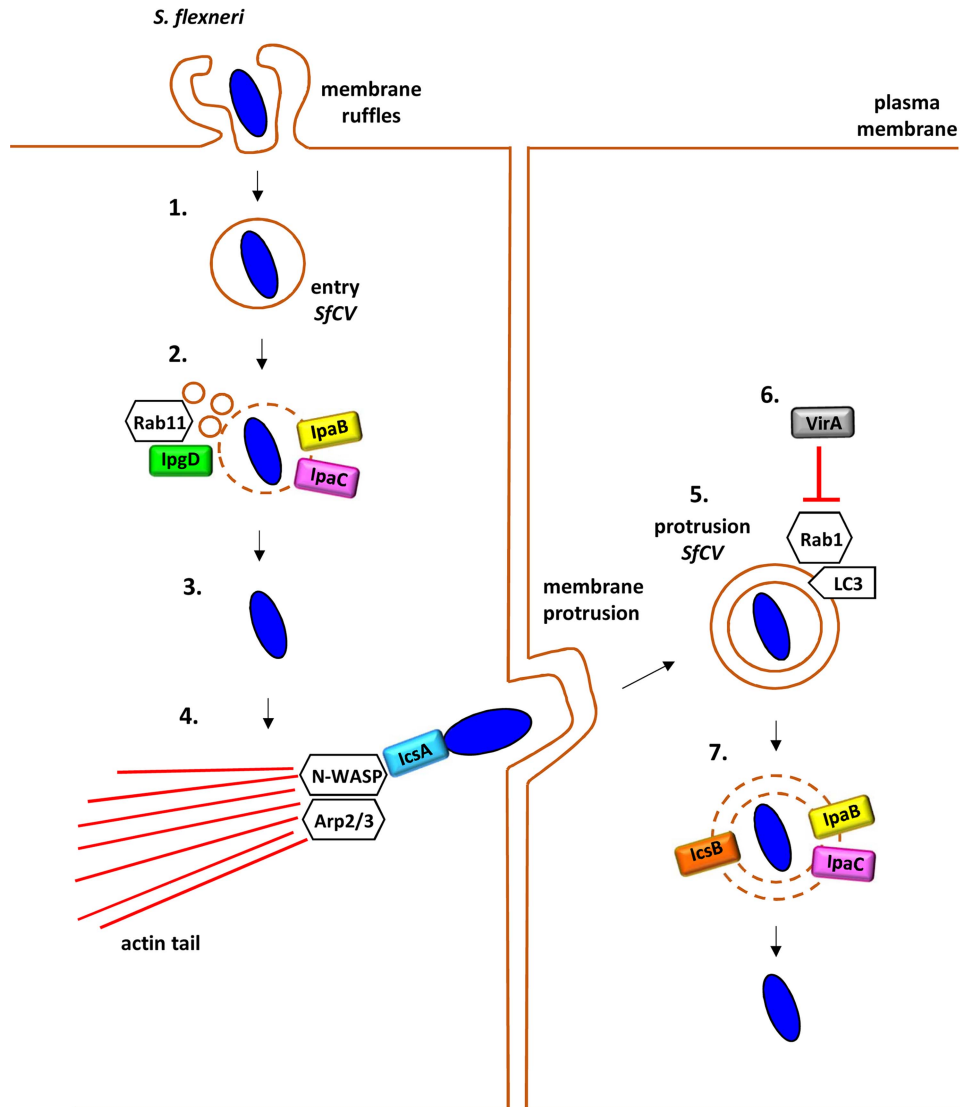
FIGURE 2 Schematic of the contribution of virulence factors to the intracellular lifecycle of *S. flexneri* **(1)**. *S. flexneri* briefly resides within its entry vacuole (*Sf*CV) **(2)**. The *Sf*CV is ruptured by the pore-forming activity of the T3SS translocon proteins IpaB and IpaC. IpgD facilitates vacuolar disruption by generating Rab11-macropinosomes that fuse to *S. flexneri* **(3)**. Upon rupture of the *Sf*CV, *Shigella* escapes into the host cytosol, from where the bacterium employs its IcsA to recruit actin cytoskeleton machinery, namely, N-WASP and Arp2/3 that polymerize actin filaments at one pole of the bacterium **(4)**. Unidirectional actin polymerization propels the bacterium across the host cytosol, leading to protrusions that enable bacterial spread into the neighboring cell **(5)**. In the secondary cell, *S. flexneri* is initially contained within a double-membrane vacuole (protrusion *Sf*CV) **(6)**. Recruitment of LC3 to the protrusion vacuole is controlled by the T3SS effector VirA, which targets the Rho GTPase Rab1 **(7)**. IpaB, IpaC, and the T3SS effector IcsB promote bacterial escape from the protrusion of *Sf*CV into the cytosol, enabling the bacterium to complete another infection cycle.

siently acquires early endosomal features, such as the EEA1 protein, and subsequently matures into a late endosome-like organelle, given by the acquisition of LAMP-1 and the acidification of its lumen ([12](#)). Luminal acidification is an important cue that triggers the bacterium to break out of its vacuole and escape into the host cytosol, where prolific bacterial replication (100 to 300 bacteria/cell) takes place ([Fig. 3](#)) ([12](#)). The bacterial

TABLE 2 Virulence factors that contribute to the intracellular lifecycle of *S. flexneri*

Effector	Secretion system	Cellular target	Biochemical activity	Biological function	References
IpgD	T3SS	PI(4,5)P$_2$	4-Phosphatase	Vacuole rupture	79–82
IpaB	T3SS	Membrane lipids	Pore-forming	Vacuole rupture	83–85, 87–89
IpaC	T3SS	Membrane lipids	Pore-forming	Vacuole rupture	84, 86–89
IcsA	T5SS/T2SS	Actin	Actin filament	Cell motility	90–98
IcsB	T3SS	Unknown/cholesterol	Unknown	Vacuole rupture	99–103
VirA	T3SS	Rab1	GTPase hydrolysis	Autophagy evasion	100, 104

factors that contribute to each of these steps remain completely unknown.

A decade went by between the genome sequencing that revealed *V. parahaemolyticus*' T3SSs and the discovery of the *V. parahaemolyticus* intracellular lifestyle (11, 107). During this period, many of the bacterium's T3SS2 effectors were characterized from the realm of *V. parahaemolyticus* being an exclusively extracellular bacterium. As a result, the cellular targets and biochemical activities of these effectors were uncovered, but the relevant roles they play during invasive infection remained unknown. An example is VopL, previously identified as a potent nucleator of actin filaments that initially was thought to induce the formation of stress fibers but later was found to catalyze the formation of nonfunctional actin linear strings (113, 114).

Analysis of the activity of VopL demonstrated that this effector plays a critical role in a process required for *V. parahaemolyticus* intracellular survival (115). This process is the assembly and activation of the NADPH oxidase enzymatic complex. The NAPDH oxidase is a major source of bactericidal reactive oxygen species (ROS) in host cells. In the absence of VopL, host epithelial cells produce ROS via NADPH oxidase that damage the DNA of cytosolic bacteria (115). As a result, *V. parahaemolyticus* exhibits erratic cell division with a resulting filamentous state and defective intracellular replication (115). VopL antagonizes bacterial deleterious events by inhibiting the actin-dependent movement of NADPH oxidase subunits to their site of complex assembly (host membranes), thereby precluding ROS generation (Fig. 3) (115). This was the first example of a T3SS effector that targets the actin cytoskeleton as a mechanism to suppress the ROS response.

The novel understanding of *V. parahaemolyticus* as an intracellular bacterium compares to a "rediscovery" of this bacterium, which presents itself as a model poised for future studies of T3SS-mediated disruption of intracellular processes. Like VopL, many of the already known T3SS2 effectors need to be reassessed with consideration of *V. parahaemolyticus*' intracellular lifecycle to reveal their relevant biological functions. Moreover,

the pathogenicity island that comprises the T3SS2 is predicted to encode additional putative effectors that likely contribute to the bacterium's intracellular lifestyle and merits investigation.

CONCLUSIONS

The coexistence of bacterial pathogens and their hosts enabled many bacteria to establish an intracellular infection as a result of convergent evolution. T3SS effectors are one of the best examples of convergent evolution, because they are often mimics of eukaryotic proteins. Mimicry of eukaryotic proteins by T3SS effectors comes in different flavors. In some instances, the mimicry is functional, as in the case of *S.* Typhimurium's SopE, which bears neither sequence nor structural homology to the Dbl family of eukaryotic GEFs of Cdc42 (116). Instead, SopE belongs to a family of bacterial WxxxE GEFs (116). Importantly, SopE and Dbl members interact with the switch I and II regions of Cdc42 in a very similar manner to facilitate nucleotide exchange (116). In other instances, T3SS effectors are homologous to eukaryotic proteins but carry out their biochemical functions in a distinctive manner. One example of this is *S.* Typhimurium SteC, a kinase that exhibits sequence similarity to eukaryotic kinases, including its closest homolog, Raf1. These kinases target the same substrate, namely MEK, but while Raf1 phosphorylates residues within the catalytic domain of MEK, SteC phosphorylates an allosteric residue, which induces a conformational change of MEK (74).

Sometimes, different bacterial effectors possess a conserved eukaryotic domain and catalyze the same biochemical reaction but play distinct biological roles. For instance, the *S.* Typhimurium effector SopB and the *S. flexneri* effector IpgD are homologs to each other and to eukaryotic PI4,5-P$_2$ phosphatases (19, 79). PI4,5-P$_2$ hydrolysis by both SopB and IpgD results in formation of macropinosomes (22, 82). Curiously, SopB-formed macropinosomes are important as a membrane source for the formation of the spacious SCV (22). IpgD-formed macropinosomes, on the other hand, promote rupture of
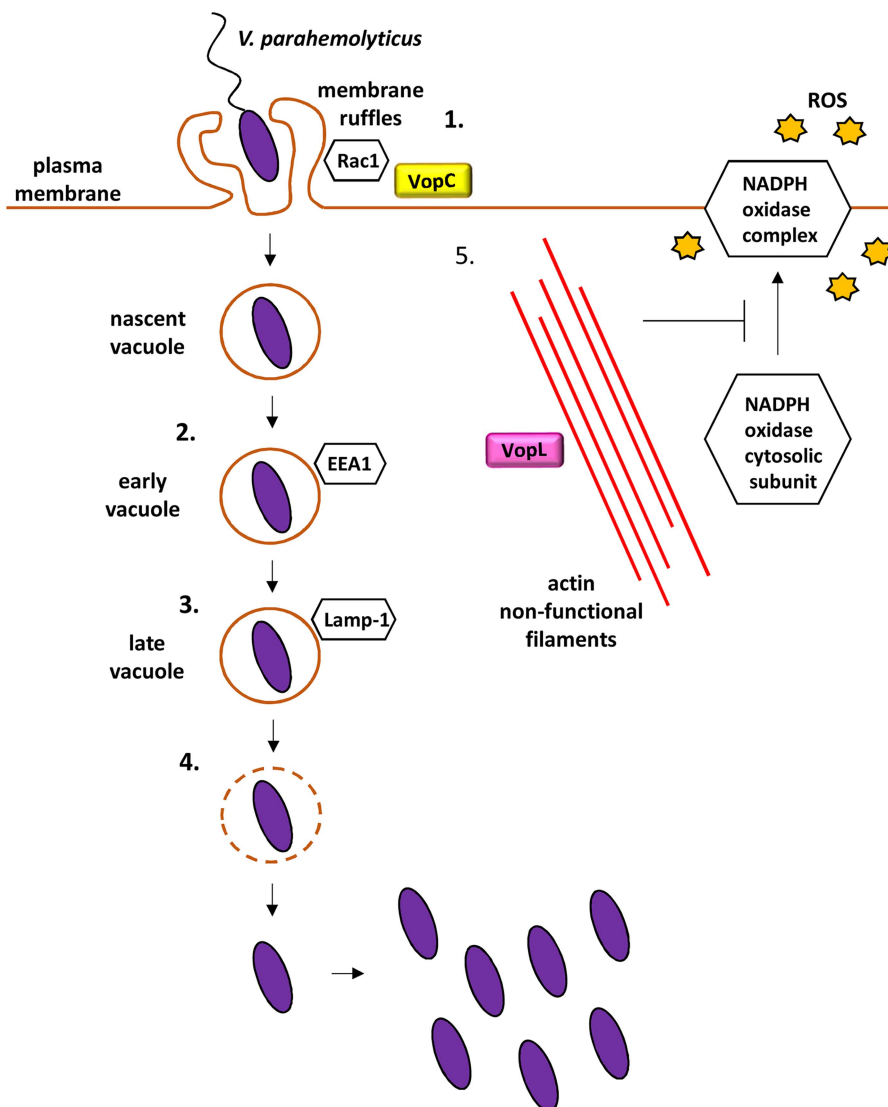
FIGURE 3 Schematic of the *V. parahaemolyticus* intracellular lifecycle and the T3SS effectors that contribute to it **(1)**. The T3SS2 effector VopC induces epithelial host cell plasma membrane ruffling that internalizes *V. parahaemolyticus* into a nascent vacuole. The nascent vacuole develops first into an early endosome-like compartment, given by its acquisition of EEA1 **(2)** and subsequently matures into a late endosome-like vacuole, given by the recruitment of Lamp-1 **(3)**. **(4)** The bacterium disrupts its containing vacuole and escapes into the cytosol, where bacterial replication takes place **(5)**. To evade host immune defenses, *V. parahaemolyticus* employs the T3SS2 VopL, which disrupts the actin cytoskeleton and thereby inhibits the actin-dependent assembly of the ROS-producing NADPH oxidase complex.

the *Shigella*-containing vacuole ([82]). Altogether, these examples underscore the extraordinary ability of bacteria to adapt protein functionalities that best suit these pathogens during infection. The study of T3SS effectors also contributes to furthering the understanding of eukaryotic cell biology. It was through the characterization of *S.* Typhimurium SifA that the protein SKIP was identified and with that, it was possible to better understand kinesin-dependent anterograde cargo trafficking ([44]).

The enteric pathogens *S.* Typhimurium, *S. flexneri*, and *V. parahaemolyticus* share many of the same cellular hosts but adopt distinct intracellular lifestyles to survive and propagate within these cells. *S.* Typhimurium, at large, resides within its crafted SCV, *S. flexneri* rapidly (~10 min) ruptures its vacuole to spread across the host cell, and *V. parahaemolyticus* maintains longer residence (~1 h) within its vacuole prior to its escape into the host cytosol. Adaptation into each of these distinct

lifestyles is largely a result of the fact that each bacterium is equipped with a unique suite of T3SS effectors, which underlines the significance of characterizing each of these systems. *S.* Typhimurium and *S. flexneri* are established, well-studied models of intracellular infection, while *V. parahaemolyticus* provides a new model for future discoveries.

ACKNOWLEDGMENTS

REFERENCES

1. **Samanta D, Mulye M, Clemente TM, Justis AV, Gilk SD.** 2017. Manipulation of host cholesterol by obligate intracellular bacteria. *Front Cell Infect Microbiol* **7:**165 http://dx.doi.org/10.3389/fcimb.2017.00165.

2. **Flint A, Butcher J, Stintzi A.** 2016. Stress responses, adaptation, and virulence of bacterial pathogens during host gastrointestinal colonization. *Microbiol Spectr* **4:**VMBF-0007-2015. doi:10.1128/microbiolspec.VMBF-0007-2015.

3. **Mellouk N, Enninga J.** 2016. Cytosolic access of intracellular bacterial pathogens: the *Shigella* paradigm. *Front Cell Infect Microbiol* **6:**35 http://dx.doi.org/10.3389/fcimb.2016.00035.

4. **Ribet D, Cossart P.** 2015. How bacterial pathogens colonize their hosts and invade deeper tissues. *Microbes Infect* **17:**173–183 http://dx.doi.org/10.1016/j.micinf.2015.01.004.

5. **Ray K, Marteyn B, Sansonetti PJ, Tang CM.** 2009. Life on the inside: the intracellular lifestyle of cytosolic bacteria. *Nat Rev Microbiol* **7:**333–340 http://dx.doi.org/10.1038/nrmicro2112.

6. **Galán JE, Waksman G.** 2018. Protein-injection machines in bacteria. *Cell* **172:**1306–1318 http://dx.doi.org/10.1016/j.cell.2018.01.034.

7. **Jennings E, Thurston TLM, Holden DW.** 2017. Salmonella SPI-2 type III secretion system effectors: molecular mechanisms and physiological consequences. *Cell Host Microbe* **22:**217–231 http://dx.doi.org/10.1016/j.chom.2017.07.009.

8. **Agaisse H.** 2016. Molecular and cellular mechanisms of *Shigella flexneri* dissemination. *Front Cell Infect Microbiol* **6:**29 http://dx.doi.org/10.3389/fcimb.2016.00029.

9. **Agbor TA, McCormick BA.** 2011. *Salmonella* effectors: important players modulating host cell function during infection. *Cell Microbiol* **13:**1858–1869 http://dx.doi.org/10.1111/j.1462-5822.2011.01701.x.

10. **Letchumanan V, Chan KG, Lee LH.** 2014. *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advance molecular identification techniques. *Front Microbiol* **5:**705 http://dx.doi.org/10.3389/fmicb.2014.00705.

11. **Zhang L, Krachler AM, Broberg CA, Li Y, Mirzaei H, Gilpin CJ, Orth K.** 2012. Type III effector VopC mediates invasion for *Vibrio* species. *Cell Rep* **1:**453–460 http://dx.doi.org/10.1016/j.celrep.2012.04.004.

12. **de Souza Santos M, Orth K.** 2014. Intracellular *Vibrio parahaemolyticus* escapes the vacuole and establishes a replicative niche in the cytosol of epithelial cells. *MBio* **5:**e01506-14 http://dx.doi.org/10.1128/mBio.01506-14.

13. **Hardt WD, Chen LM, Schuebel KE, Bustelo XR, Galán JE.** 1998. *S. typhimurium* encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* **93:**815–826 http://dx.doi.org/10.1016/S0092-8674(00)81442-7.

14. **Stender S, Friebel A, Linder S, Rohde M, Mirold S, Hardt WD.** 2000. Identification of SopE2 from *Salmonella typhimurium*, a conserved guanine nucleotide exchange factor for Cdc42 of the host cell. *Mol Microbiol* **36:**1206–1221 http://dx.doi.org/10.1046/j.1365-2958.2000.01933.x.

15. **Humphreys D, Davidson A, Hume PJ, Koronakis V.** 2012. *Salmonella* virulence effector SopE and host GEF ARNO cooperate to recruit and activate WAVE to trigger bacterial invasion. *Cell Host Microbe* **11:**129–139 http://dx.doi.org/10.1016/j.chom.2012.01.006.

16. **Kubori T, Galán JE.** 2003. Temporal regulation of *Salmonella* virulence effector function by proteasome-dependent protein degradation. *Cell* **115:**333–342 http://dx.doi.org/10.1016/S0092-8674(03)00849-3.

17. **Vonaesch P, Sellin ME, Cardini S, Singh V, Barthel M, Hardt WD.** 2014. The *Salmonella* Typhimurium effector protein SopE transiently localizes to the early SCV and contributes to intracellular replication. *Cell Microbiol* **16:**1723–1735 http://dx.doi.org/10.1111/cmi.12333.

18. **Mukherjee K, Parashuraman S, Raje M, Mukhopadhyay A.** 2001. SopE acts as an Rab5-specific nucleotide exchange factor and recruits nonprenylated Rab5 on *Salmonella*-containing phagosomes to promote fusion with early endosomes. *J Biol Chem* **276:**23607–23615 http://dx.doi.org/10.1074/jbc.M101034200.

19. **Norris FA, Wilson MP, Wallis TS, Galyov EE, Majerus PW.** 1998. SopB, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase. *Proc Natl Acad Sci U S A* **95:**14057–14059 http://dx.doi.org/10.1073/pnas.95.24.14057.

20. **Terebiznik MR, Vieira OV, Marcus SL, Slade A, Yip CM, Trimble WS, Meyer T, Finlay BB, Grinstein S.** 2002. Elimination of host cell PtdIns (4,5)P(2) by bacterial SigD promotes membrane fission during invasion by *Salmonella*. *Nat Cell Biol* **4:**766–773 http://dx.doi.org/10.1038/ncb854.

21. **Scott CC, Cuellar-Mata P, Matsuo T, Davidson HW, Grinstein S.** 2002. Role of 3-phosphoinositides in the maturation of *Salmonella*-containing vacuoles within host cells. *J Biol Chem* **277:**12770–12776 http://dx.doi.org/10.1074/jbc.M110399200.

22. **Hernandez LD, Hueffer K, Wenk MR, Galán JE.** 2004. *Salmonella* modulates vesicular traffic by altering phosphoinositide metabolism. *Science* **304:**1805–1807 http://dx.doi.org/10.1126/science.1098188.

23. **Mallo GV, Espina M, Smith AC, Terebiznik MR, Alemán A, Finlay BB, Rameh LE, Grinstein S, Brumell JH.** 2008. SopB promotes phosphatidylinositol 3-phosphate formation on *Salmonella* vacuoles by recruiting Rab5 and Vps34. *J Cell Biol* **182:**741–752 http://dx.doi.org/10.1083/jcb.200804131.

24. **Méresse S, Steele-Mortimer O, Finlay BB, Gorvel JP.** 1999. The rab7 GTPase controls the maturation of *Salmonella typhimurium*-containing vacuoles in HeLa cells. *EMBO J* **18:**4394–4403 http://dx.doi.org/10.1093/emboj/18.16.4394.

25. **Guerra F, Bucci C.** 2016. Multiple roles of the small GTPase Rab7. *Cells* **5:**E34 http://dx.doi.org/10.3390/cells5030034.

26. **Ramsden AE, Mota LJ, Münter S, Shorte SL, Holden DW.** 2007. The SPI-2 type III secretion system restricts motility of *Salmonella*-containing vacuoles. *Cell Microbiol* **9:**2517–2529 http://dx.doi.org/10.1111/j.1462-5822.2007.00977.x.

27. **Salcedo SP, Holden DW.** 2003. SseG, a virulence protein that targets *Salmonella* to the Golgi network. *EMBO J* **22:**5003–5014 http://dx.doi.org/10.1093/emboj/cdg517.

28. **D'Costa VM, Braun V, Landekic M, Shi R, Proteau A, McDonald L, Cygler M, Grinstein S, Brumell JH.** 2015. *Salmonella* disrupts host endocytic trafficking by SopD2-mediated inhibition of Rab7. *Cell Rep* **12:**1508–1518 http://dx.doi.org/10.1016/j.celrep.2015.07.063.

29. **Spanò S, Galán JE.** 2012. A Rab32-dependent pathway contributes to *Salmonella typhi* host restriction. *Science* **338:**960–963 http://dx.doi.org/10.1126/science.1229224.

30. **Spanò S, Liu X, Galán JE.** 2011. Proteolytic targeting of Rab29 by an effector protein distinguishes the intracellular compartments of human-

adapted and broad-host *Salmonella*. *Proc Natl Acad Sci U S A* **108:** 18418–18423 http://dx.doi.org/10.1073/pnas.1111959108.

31. **Kohler AC, Spanò S, Galán JE, Stebbins CE.** 2014. Structural and enzymatic characterization of a host-specificity determinant from *Salmonella*. *Acta Crystallogr D Biol Crystallogr* **70:**384–391 http://dx.doi.org /10.1107/S1399004713028393.

32. **Wachtel R, Bräuning B, Mader SL, Ecker F, Kaila VRI, Groll M, Itzen A.** 2018. The protease GtgE from *Salmonella* exclusively targets inactive Rab GTPases. *Nat Commun* **9:**44 http://dx.doi.org/10.1038/s41467-017 -02110-1.

33. **Ohbayashi N, Fukuda M, Kanaho Y.** 2017. Rab32 subfamily small GTPases: pleiotropic Rabs in endosomal trafficking. *J Biochem* **162:**65–71 http://dx.doi.org/10.1093/jb/mvx027.

34. **Spanò S, Gao X, Hannemann S, Lara-Tejero M, Galán JE.** 2016. A bacterial pathogen targets a host Rab-family GTPase defense pathway with a GAP. *Cell Host Microbe* **19:**216–226 http://dx.doi.org/10.1016/j .chom.2016.01.004.

35. **Bujny MV, Ewels PA, Humphrey S, Attar N, Jepson MA, Cullen PJ.** 2008. Sorting nexin-1 defines an early phase of *Salmonella*-containing vacuole-remodeling during *Salmonella* infection. *J Cell Sci* **121:**2027– 2036 http://dx.doi.org/10.1242/jcs.018432.

36. **Bakowski MA, Braun V, Lam GY, Yeung T, Heo WD, Meyer T, Finlay BB, Grinstein S, Brumell JH.** 2010. The phosphoinositide phosphatase SopB manipulates membrane surface charge and trafficking of the *Salmonella*-containing vacuole. *Cell Host Microbe* **7:**453–462 http://dx .doi.org/10.1016/j.chom.2010.05.011.

37. **Kuhle V, Jäckel D, Hensel M.** 2004. Effector proteins encoded by *Salmonella* pathogenicity island 2 interfere with the microtubule cytoskeleton after translocation into host cells. *Traffic* **5:**356–370 http://dx .doi.org/10.1111/j.1398-9219.2004.00179.x.

38. **Abrahams GL, Müller P, Hensel M.** 2006. Functional dissection of SseF, a type III effector protein involved in positioning the *Salmonella*-containing vacuole. *Traffic* **7:**950–965 http://dx.doi.org/10.1111/j.1600 -0854.2006.00454.x.

39. **Deiwick J, Salcedo SP, Boucrot E, Gilliland SM, Henry T, Petermann N, Waterman SR, Gorvel JP, Holden DW, Méresse S.** 2006. The translocated *Salmonella* effector proteins SseF and SseG interact and are required to establish an intracellular replication niche. *Infect Immun* **74:** 6965–6972 http://dx.doi.org/10.1128/IAI.00648-06.

40. **Yu XJ, Liu M, Holden DW.** 2016. *Salmonella* effectors SseF and SseG interact with mammalian protein ACBD3 (GCP60) to anchor *Salmonella*-containing vacuoles at the Golgi network. *MBio* **7:**e00474-16 http://dx -doi.org/10.1128/mBio.00474-16.

41. **Mallik R, Rai AK, Barak P, Rai A, Kunwar A.** 2013. Teamwork in microtubule motors. *Trends Cell Biol* **23:**575–582 http://dx.doi.org/10 .1016/j.tcb.2013.06.003.

42. **Knodler LA, Steele-Mortimer O.** 2005. The *Salmonella* effector PipB2 affects late endosome/lysosome distribution to mediate Sif extension. *Mol Biol Cell* **16:**4108–4123 http://dx.doi.org/10.1091/mbc.e05-04-0367.

43. **Henry T, Couillault C, Rockenfeller P, Boucrot E, Dumont A, Schroeder N, Hermant A, Knodler LA, Lecine P, Steele-Mortimer O, Borg JP, Gorvel JP, Méresse S.** 2006. The *Salmonella* effector protein PipB2 is a linker for kinesin-1. *Proc Natl Acad Sci U S A* **103:**13497–13502 http:// dx.doi.org/10.1073/pnas.0605443103.

44. **Boucrot E, Henry T, Borg JP, Gorvel JP, Méresse S.** 2005. The intracellular fate of *Salmonella* depends on the recruitment of kinesin. *Science* **308:**1174–1178 http://dx.doi.org/10.1126/science.1110225.

45. **Zhou D, Mooseker MS, Galán JE.** 1999. Role of the *S. typhimurium* actin-binding protein SipA in bacterial internalization. *Science* **283:**2092– 2095 http://dx.doi.org/10.1126/science.283.5410.2092.

46. **Zhou D, Mooseker MS, Galán JE.** 1999. An invasion-associated *Salmonella* protein modulates the actin-bundling activity of plastin. *Proc Natl Acad Sci U S A* **96:**10176–10181 http://dx.doi.org/10.1073/pnas .96.18.10176.

47. **McGhie EJ, Hayward RD, Koronakis V.** 2004. Control of actin turnover by a *Salmonella* invasion protein. *Mol Cell* **13:**497–510 http:// dx.doi.org/10.1016/S1097-2765(04)00053-X.

48. **Brawn LC, Hayward RD, Koronakis V.** 2007. *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 http://dx.doi.org/10.1016/j.chom.2007.02.001.

49. **Garcia-del Portillo F, Zwick MB, Leung KY, Finlay BB.** 1993. *Salmonella* induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells. *Proc Natl Acad Sci U S A* **90:**10544–10548 http://dx.doi.org/10.1073/pnas.90.22.10544.

50. **Garcia-del Portillo F, Zwick MB, Leung KY, Finlay BB.** 1993. Intracellular replication of *Salmonella* within epithelial cells is associated with filamentous structures containing lysosomal membrane glycoproteins. *Infect Agents Dis* **2:**227–231.

51. **Stein MA, Leung KY, Zwick M, Garcia-del Portillo F, Finlay BB.** 1996. Identification of a *Salmonella* virulence gene required for formation of filamentous structures containing lysosomal membrane glycoproteins within epithelial cells. *Mol Microbiol* **20:**151–164 http://dx.doi.org/10 .1111/j.1365-2958.1996.tb02497.x.

52. **Brumell JH, Goosney DL, Finlay BB.** 2002. SifA, a type III secreted effector of *Salmonella typhimurium*, directs *Salmonella*-induced filament (Sif) formation along microtubules. *Traffic* **3:**407–415 http://dx.doi.org /10.1034/j.1600-0854.2002.30604.x.

53. **Dumont A, Boucrot E, Drevensek S, Daire V, Gorvel JP, Poüs C, Holden DW, Méresse S.** 2010. SKIP, the host target of the *Salmonella* virulence factor SifA, promotes kinesin-1-dependent vacuolar membrane exchanges. *Traffic* **11:**899–911 http://dx.doi.org/10.1111/j.1600-0854.2010 .01069.x.

54. **Harrison RE, Brumell JH, Khandani A, Bucci C, Scott CC, Jiang X, Finlay BB, Grinstein S.** 2004. *Salmonella* impairs RILP recruitment to Rab7 during maturation of invasion vacuoles. *Mol Biol Cell* **15:**3146– 3154 http://dx.doi.org/10.1091/mbc.e04-02-0092.

55. **Fu Y, Galán JE.** 1999. A *Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. *Nature* **401:**293–297 http://dx.doi.org/10.1038/45829.

56. **Humphreys D, Hume PJ, Koronakis V.** 2009. The *Salmonella* effector SptP dephosphorylates host AAA+ ATPase VCP to promote development of its intracellular replicative niche. *Cell Host Microbe* **5:**225–233 http:// dx.doi.org/10.1016/j.chom.2009.01.010.

57. **Kaniga K, Uralil J, Bliska JB, Galán JE.** 1996. A secreted protein tyrosine phosphatase with modular effector domains in the bacterial pathogen *Salmonella* typhimurium. *Mol Microbiol* **21:**633–641 http://dx .doi.org/10.1111/j.1365-2958.1996.tb02571.x.

58. **McGourty K, Thurston TL, Matthews SA, Pinaud L, Mota LJ, Holden DW.** 2012. *Salmonella* inhibits retrograde trafficking of mannose-6-phosphate receptors and lysosome function. *Science* **338:**963–967 http:// dx.doi.org/10.1126/science.1227037.

59. **Sindhwani A, Arya SB, Kaur H, Jagga D, Tuli A, Sharma M.** 2017. *Salmonella* exploits the host endolysosomal tethering factor HOPS complex to promote its intravacuolar replication. *PLoS Pathog* **13:**e1006700 http://dx.doi.org/10.1371/journal.ppat.1006700.

60. **Beuzón CR, Méresse S, Unsworth KE, Ruíz-Albert J, Garvis S, Waterman SR, Ryder TA, Boucrot E, Holden DW.** 2000. *Salmonella* maintains the integrity of its intracellular vacuole through the action of SifA. *EMBO J* **19:**3235–3249 http://dx.doi.org/10.1093/emboj/19.13.3235.

61. **Ruiz-Albert J, Yu XJ, Beuzón CR, Blakey AN, Galyov EE, Holden DW.** 2002. Complementary activities of SseJ and SifA regulate dynamics of the *Salmonella typhimurium* vacuolar membrane. *Mol Microbiol* **44:** 645–661 http://dx.doi.org/10.1046/j.1365-2958.2002.02912.x.

62. **Ohlson MB, Fluhr K, Birmingham CL, Brumell JH, Miller SI.** 2005. SseJ deacylase activity by *Salmonella enterica* serovar Typhimurium promotes virulence in mice. *Infect Immun* **73:**6249–6259 http://dx.doi.org /10.1128/IAI.73.10.6249-6259.2005.

63. Nawabi P, Catron DM, Haldar K. 2008. Esterification of cholesterol by a type III secretion effector during intracellular *Salmonella* infection. *Mol Microbiol* **68:**173–185 http://dx.doi.org/10.1111/j.1365-2958.2008.06142.x.

64. Lossi NS, Rolhion N, Magee AI, Boyle C, Holden DW. 2008. The *Salmonella* SPI-2 effector SseJ exhibits eukaryotic activator-dependent phospholipase A and glycerophospholipid : cholesterol acyltransferase activity. *Microbiology* **154:**2680–2688 http://dx.doi.org/10.1099/mic.0.2008/019075-0.

65. Christen M, Coye LH, Hontz JS, LaRock DL, Pfuetzner RA, Megha, Miller SI. 2009. Activation of a bacterial virulence protein by the GTPase RhoA. *Sci Signal* **2:**ra71 http://dx.doi.org/10.1126/scisignal.2000430.

66. LaRock DL, Brzovic PS, Levin I, Blanc MP, Miller SI. 2012. A *Salmonella typhimurium*-translocated glycerophospholipid:cholesterol acyltransferase promotes virulence by binding to the RhoA protein switch regions. *J Biol Chem* **287:**29654–29663 http://dx.doi.org/10.1074/jbc.M112.363598.

67. Ohlson MB, Huang Z, Alto NM, Blanc MP, Dixon JE, Chai J, Miller SI. 2008. Structure and function of *Salmonella* SifA indicate that its interactions with SKIP, SseJ, and RhoA family GTPases induce endosomal tubulation. *Cell Host Microbe* **4:**434–446 http://dx.doi.org/10.1016/j.chom.2008.08.012.

68. Geddes K, Worley M, Niemann G, Heffron F. 2005. Identification of new secreted effectors in *Salmonella enterica* serovar Typhimurium. *Infect Immun* **73:**6260–6271 http://dx.doi.org/10.1128/IAI.73.10.6260-6271.2005.

69. Cardenal-Muñoz E, Ramos-Morales F. 2011. Analysis of the expression, secretion and translocation of the *Salmonella enterica* type III secretion system effector SteA. *PLoS One* **6:**e26930 http://dx.doi.org/10.1371/journal.pone.0026930.

70. Domingues L, Ismail A, Charro N, Rodríguez-Escudero I, Holden DW, Molina M, Cid VJ, Mota LJ. 2016. The *Salmonella* effector SteA binds phosphatidylinositol 4-phosphate for subcellular targeting within host cells. *Cell Microbiol* **18:**949–969 http://dx.doi.org/10.1111/cmi.12558.

71. Domingues L, Holden DW, Mota LJ. 2014. The *Salmonella* effector SteA contributes to the control of membrane dynamics of *Salmonella*-containing vacuoles. *Infect Immun* **82:**2923–2934 http://dx.doi.org/10.1128/IAI.01385-13.

72. Méresse S, Unsworth KE, Habermann A, Griffiths G, Fang F, Martínez-Lorenzo MJ, Waterman SR, Gorvel JP, Holden DW. 2001. Remodelling of the actin cytoskeleton is essential for replication of intravacuolar *Salmonella*. *Cell Microbiol* **3:**567–577 http://dx.doi.org/10.1046/j.1462-5822.2001.00141.x.

73. Poh J, Odendall C, Spanos A, Boyle C, Liu M, Freemont P, Holden DW. 2008. SteC is a *Salmonella* kinase required for SPI-2-dependent F-actin remodelling. *Cell Microbiol* **10:**20–30.

74. Odendall C, Rolhion N, Förster A, Poh J, Lamont DJ, Liu M, Freemont PS, Catling AD, Holden DW. 2012. The *Salmonella* kinase SteC targets the MAP kinase MEK to regulate the host actin cytoskeleton. *Cell Host Microbe* **12:**657–668 http://dx.doi.org/10.1016/j.chom.2012.09.011.

75. Tezcan-Merdol D, Nyman T, Lindberg U, Haag F, Koch-Nolte F, Rhen M. 2001. Actin is ADP-ribosylated by the *Salmonella enterica* virulence-associated protein SpvB. *Mol Microbiol* **39:**606–619 http://dx.doi.org/10.1046/j.1365-2958.2001.02258.x.

76. Lesnick ML, Reiner NE, Fierer J, Guiney DG. 2001. The *Salmonella* spvB virulence gene encodes an enzyme that ADP-ribosylates actin and destabilizes the cytoskeleton of eukaryotic cells. *Mol Microbiol* **39:**1464–1470 http://dx.doi.org/10.1046/j.1365-2958.2001.02360.x.

77. Miao EA, Brittnacher M, Haraga A, Jeng RL, Welch MD, Miller SI. 2003. *Salmonella* effectors translocated across the vacuolar membrane interact with the actin cytoskeleton. *Mol Microbiol* **48:**401–415 http://dx.doi.org/10.1046/j.1365-2958.2003.t01-1-03456.x.

78. Mattock E, Blocker AJ. 2017. How do the virulence factors of *Shigella* work together to cause disease? *Front Cell Infect Microbiol* **7:**64 http://dx.doi.org/10.3389/fcimb.2017.00064.

79. Niebuhr K, Giuriato S, Pedron T, Philpott DJ, Gaits F, Sable J, Sheetz MP, Parsot C, Sansonetti PJ, Payrastre B. 2002. 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 http://dx.doi.org/10.1093/emboj/cdf522.

80. Weiner A, Mellouk N, Lopez-Montero N, Chang YY, Souque C, Schmitt C, Enninga J. 2016. Macropinosomes are key players in early *Shigella* invasion and vacuolar escape in epithelial cells. *PLoS Pathog* **12:**e1005602 http://dx.doi.org/10.1371/journal.ppat.1005602.

81. Allaoui A, Ménard R, Sansonetti PJ, Parsot C. 1993. Characterization of the *Shigella flexneri* ipgD and ipgF genes, which are located in the proximal part of the mxi locus. *Infect Immun* **61:**1707–1714.

82. Mellouk N, Weiner A, Aulner N, Schmitt C, Elbaum M, Shorte SL, Danckaert A, Enninga J. 2014. *Shigella* subverts the host recycling compartment to rupture its vacuole. *Cell Host Microbe* **16:**517–530 http://dx.doi.org/10.1016/j.chom.2014.09.005.

83. De Geyter C, Wattiez R, Sansonetti P, Falmagne P, Ruysschaert JM, Parsot C, Cabiaux V. 2000. Characterization of the interaction of IpaB and IpaD, proteins required for entry of *Shigella flexneri* into epithelial cells, with a lipid membrane. *Eur J Biochem* **267:**5769–5776 http://dx.doi.org/10.1046/j.1432-1327.2000.01649.x.

84. Veenendaal AK, Hodgkinson JL, Schwarzer L, Stabat D, Zenk SF, Blocker AJ. 2007. The type III secretion system needle tip complex mediates host cell sensing and translocon insertion. *Mol Microbiol* **63:**1719–1730 http://dx.doi.org/10.1111/j.1365-2958.2007.05620.x.

85. High N, Mounier J, Prévost MC, Sansonetti PJ. 1992. IpaB of *Shigella flexneri* causes entry into epithelial cells and escape from the phagocytic vacuole. *EMBO J* **11:**1991–1999 http://dx.doi.org/10.1002/j.1460-2075.1992.tb05253.x.

86. Bârzu S, Benjelloun-Touimi Z, Phalipon A, Sansonetti P, Parsot C. 1997. Functional analysis of the *Shigella flexneri* IpaC invasin by insertional mutagenesis. *Infect Immun* **65:**1599–1605.

87. Page AL, Ohayon H, Sansonetti PJ, Parsot C. 1999. The secreted IpaB and IpaC invasins and their cytoplasmic chaperone IpgC are required for intercellular dissemination of *Shigella flexneri*. *Cell Microbiol* **1:**183–193 http://dx.doi.org/10.1046/j.1462-5822.1999.00019.x.

88. Schuch R, Sandlin RC, Maurelli AT. 1999. A system for identifying post-invasion functions of invasion genes: requirements for the Mxi-Spa type III secretion pathway of *Shigella flexneri* in intercellular dissemination. *Mol Microbiol* **34:**675–689 http://dx.doi.org/10.1046/j.1365-2958.1999.01627.x.

89. Du J, Reeves AZ, Klein JA, Twedt DJ, Knodler LA, Lesser CF. 2016. The type III secretion system apparatus determines the intracellular niche of bacterial pathogens. *Proc Natl Acad Sci U S A* **113:**4794–4799 http://dx.doi.org/10.1073/pnas.1520699113.

90. Bernardini ML, Mounier J, d'Hauteville H, Coquis-Rondon M, Sansonetti PJ. 1989. Identification of icsA, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc Natl Acad Sci U S A* **86:**3867–3871 http://dx.doi.org/10.1073/pnas.86.10.3867.

91. Brandon LD, Goehring N, Janakiraman A, Yan AW, Wu T, Beckwith J, Goldberg MB. 2003. IcsA, a polarly localized autotransporter with an atypical signal peptide, uses the Sec apparatus for secretion, although the Sec apparatus is circumferentially distributed. *Mol Microbiol* **50:**45–60 http://dx.doi.org/10.1046/j.1365-2958.2003.03674.x.

92. Goldberg MB, Bârzu O, Parsot C, Sansonetti PJ. 1993. Unipolar localization and ATPase activity of IcsA, a *Shigella flexneri* protein involved in intracellular movement. *J Bacteriol* **175:**2189–2196 http://dx.doi.org/10.1128/jb.175.8.2189-2196.1993.

93. Robbins JR, Monack D, McCallum SJ, Vegas A, Pham E, Goldberg MB, Theriot JA. 2001. The making of a gradient: IcsA (VirG) polarity in

Shigella flexneri. Mol Microbiol **41:**861–872 http://dx.doi.org/10.1046/j.1365-2958.2001.02552.x.

94. **Mauricio RP, Jeffries CM, Svergun DI, Deane JE.** 2017. The *Shigella* virulence factor IcsA relieves N-WASP autoinhibition by displacing the verprolin homology/cofilin/acidic (VCA) domain. *J Biol Chem* **292:**134–145 http://dx.doi.org/10.1074/jbc.M116.758003.

95. **Egile C, Loisel TP, Laurent V, Li R, Pantaloni D, Sansonetti PJ, Carlier MF.** 1999. Activation of the CDC42 effector N-WASP by the *Shigella flexneri* IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. *J Cell Biol* **146:**1319–1332 http://dx.doi.org/10.1083/jcb.146.6.1319.

96. **Suzuki T, Mimuro H, Suetsugu S, Miki H, Takenawa T, Sasakawa C.** 2002. Neural Wiskott-Aldrich syndrome protein (N-WASP) is the specific ligand for *Shigella* VirG among the WASP family and determines the host cell type allowing actin-based spreading. *Cell Microbiol* **4:**223–233 http://dx.doi.org/10.1046/j.1462-5822.2002.00185.x.

97. **Rohatgi R, Ma L, Miki H, Lopez M, Kirchhausen T, Takenawa T, Kirschner MW.** 1999. The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97:**221–231 http://dx.doi.org/10.1016/S0092-8674(00)80732-1.

98. **Goldberg MB, Theriot JA.** 1995. *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proc Natl Acad Sci U S A* **92:**6572–6576 http://dx.doi.org/10.1073/pnas.92.14.6572.

99. **Allaoui A, Mounier J, Prévost MC, Sansonetti PJ, Parsot C.** 1992. icsB: a *Shigella flexneri* virulence gene necessary for the lysis of protrusions during intercellular spread. *Mol Microbiol* **6:**1605–1616 http://dx.doi.org/10.1111/j.1365-2958.1992.tb00885.x.

100. **Campbell-Valois FX, Sachse M, Sansonetti PJ, Parsot C.** 2015. Escape of actively secreting *Shigella flexneri* from ATG8/LC3-positive vacuoles formed during cell-to-cell spread is facilitated by IcsB and VirA. *MBio* **6:**e02567-14 http://dx.doi.org/10.1128/mBio.02567-14.

101. **Weddle E, Agaisse H.** 2018. Spatial, temporal, and functional assessment of LC3-dependent autophagy in *Shigella flexneri* dissemination. *Infect Immun* **86:**e00134-18 http://dx.doi.org/10.1128/IAI.00134-18.

102. **Kayath CA, Hussey S, El hajjami N, Nagra K, Philpott D, Allaoui A.** 2010. Escape of intracellular *Shigella* from autophagy requires binding to cholesterol through the type III effector, IcsB. *Microbes Infect* **12:**956–966 http://dx.doi.org/10.1016/j.micinf.2010.06.006.

103. **Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C.** 2005. Escape of intracellular *Shigella* from autophagy. *Science* **307:**727–731 http://dx.doi.org/10.1126/science.1106036.

104. **Dong N, Zhu Y, Lu Q, Hu L, Zheng Y, Shao F.** 2012. Structurally distinct bacterial TBC-like GAPs link Arf GTPase to Rab1 inactivation to counteract host defenses. *Cell* **150:**1029–1041 http://dx.doi.org/10.1016/j.cell.2012.06.050.

105. **Zoppino FC, Militello RD, Slavin I, Alvarez C, Colombo MI.** 2010. Autophagosome formation depends on the small GTPase Rab1 and func-tional ER exit sites. *Traffic* **11:**1246–1261 http://dx.doi.org/10.1111/j.1600-0854.2010.01086.x.

106. **Shinoda S.** 2011. Sixty years from the discovery of *Vibrio parahaemolyticus* and some recollections. *Biocontrol Sci* **16:**129–137 http://dx.doi.org/10.4265/bio.16.129.

107. **Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, Tagomori K, Iijima Y, Najima M, Nakano M, Yamashita A, Kubota Y, Kimura S, Yasunaga T, Honda T, Shinagawa H, Hattori M, Iida T.** 2003. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of V cholerae. *Lancet* **361:**743–749 http://dx.doi.org/10.1016/S0140-6736(03)12659-1.

108. **Ritchie JM, Rui H, Zhou X, Iida T, Kodoma T, Ito S, Davis BM, Bronson RT, Waldor MK.** 2012. Inflammation and disintegration of intestinal villi in an experimental model for *Vibrio parahaemolyticus*-induced diarrhea. *PLoS Pathog* **8:**e1002593 http://dx.doi.org/10.1371/journal.ppat.1002593.

109. **Zhou X, Shah DH, Konkel ME, Call DR.** 2008. Type III secretion system 1 genes in *Vibrio parahaemolyticus* are positively regulated by ExsA and negatively regulated by ExsD. *Mol Microbiol* **69:**747–764 http://dx.doi.org/10.1111/j.1365-2958.2008.06326.x.

110. **Park KS, Ono T, Rokuda M, Jang MH, Okada K, Iida T, Honda T.** 2004. Functional characterization of two type III secretion systems of *Vibrio parahaemolyticus. Infect Immun* **72:**6659–6665 http://dx.doi.org/10.1128/IAI.72.11.6659-6665.2004.

111. **Burdette DL, Yarbrough ML, Orvedahl A, Gilpin CJ, Orth K.** 2008. *Vibrio parahaemolyticus* orchestrates a multifaceted host cell infection by induction of autophagy, cell rounding, and then cell lysis. *Proc Natl Acad Sci U S A* **105:**12497–12502 http://dx.doi.org/10.1073/pnas.0802773105.

112. **Salomon D, Guo Y, Kinch LN, Grishin NV, Gardner KH, Orth K.** 2013. Effectors of animal and plant pathogens use a common domain to bind host phosphoinositides. *Nat Commun* **4:**2973 http://dx.doi.org/10.1038/ncomms3973.

113. **Liverman AD, Cheng HC, Trosky JE, Leung DW, Yarbrough ML, Burdette DL, Rosen MK, Orth K.** 2007. Arp2/3-independent assembly of actin by *Vibrio* type III effector VopL. *Proc Natl Acad Sci U S A* **104:**17117–17122 http://dx.doi.org/10.1073/pnas.0703196104.

114. **Avvaru BS, Pernier J, Carlier MF.** 2015. Dimeric WH2 repeats of VopF sequester actin monomers into non-nucleating linear string conformations: an X-ray scattering study. *J Struct Biol* **190:**192–199 http://dx.doi.org/10.1016/j.jsb.2015.03.008.

115. **de Souza Santos M, Salomon D, Orth K.** 2017. T3SS effector VopL inhibits the host ROS response, promoting the intracellular survival of *Vibrio parahaemolyticus. PLoS Pathog* **13:**e1006438 http://dx.doi.org/10.1371/journal.ppat.1006438.

116. **Huang Z, Sutton SE, Wallenfang AJ, Orchard RC, Wu X, Feng Y, Chai J, Alto NM.** 2009. Structural insights into host GTPase isoform selection by a family of bacterial GEF mimics. *Nat Struct Mol Biol* **16:**853–860 http://dx.doi.org/10.1038/nsmb.1647.