

# Intracellular Growth of Bacterial Pathogens: The Role of Secreted Effector Proteins in the Control of Phagocytosed Microorganisms

# 24

VALÉRIE POIRIER<sup>1</sup> and YOSSEF AV-GAY<sup>1</sup>

## INTRODUCTION

Of the 56 million deaths reported worldwide in 2012, approximately 15 million are directly related to infectious diseases (1). The majority of annual deaths are related to bacterial infections such as tuberculosis, yellow and typhoid fever, cholera, shigellosis, pneumonia, etc. (1). Morbidity and mortality rates are highest in developing countries, where large numbers of infants and children count among the victims (2). In developed nations, infectious disease mortality falls most heavily on indigenous and disadvantaged minorities (3). The control of bacterial infectious diseases worldwide is an important task. Although antibiotics revolutionized the treatment of bacterial infections, increased resistance and the emergence of multidrug-resistant strains increasingly reduce their efficacy. This trend promotes an urgent need for better understanding of bacterial pathogenicity and resistance mechanisms, which will assist novel therapeutic and vaccination strategies.

To avoid destruction by host cells, a variety of evolutionarily unrelated bacteria have developed strategies to grow and replicate inside the host. These infectious bacteria are designated as intracellular pathogens and manipulate host responses to their advantage in unique ways. A widespread bacterial

<sup>1</sup>Division of Infectious Diseases, Department of Medicine, University of British Columbia, Vancouver, BC V6H 3Z6 Canada.

*Virulence Mechanisms of Bacterial Pathogens, 5th edition*

Edited by Indira T. Kudva, Nancy A. Cornick, Paul J. Plummer, Qijing Zhang, Tracy L. Nicholson, John P. Bannantine, and Bryan H. Bellaire

© 2016 American Society for Microbiology, Washington, DC  
doi:10.1128/microbiolspec.VMBF-0003-2014

pathogenesis trait is the synthesis and secretion of numerous proteins into the cytoplasm or membrane of the host via specialized secretion systems. These secreted macromolecules, referred to as virulence factors or effectors, facilitate bacterial pathogenesis by manipulating host cellular processes to enhance bacterial colonization and survival within the infected host, and suppress host cell defenses (4–7).

## BACTERIAL SECRETION SYSTEMS

The export of bacterial effectors occurs via secretion systems which are specialized protein translocation systems that enable transport of substrate molecules after production within the bacterium (8). These systems mediate intraspecies passage of genetic material, such as antibiotic resistance genes, as well as transfer of virulence factors across cellular membranes into the cytoplasm of the host. Consequently, secretion systems facilitate aspects of the infection process such as bacterial entry into the host cell, intracellular survival, and spreading of the pathogen to neighboring host cells (9).

These secretion systems have been categorized into six evolutionary and functionally related groups, namely type I to VI secretion systems (10). However, some species of Gram-positive bacteria use alternative protein secretion systems collectively called the type VII secretion system (10). The emphasis of this chapter is placed on bacterial macromolecules involved in manipulating phagosomal trafficking that are secreted by the membrane-associated transporter complexes type III, IV, and VII secretion systems. However, although not discussed in detail, we note that *Listeria* species export virulence factors via a general secretory pathway, the SecA2 secretion system (11).

### Type III, IV, and VII Secretion Systems

Gram-negative bacteria possess a cell envelope composed of two membranes: an inner

and an outer membrane (12). To manipulate host cells, these bacteria have developed an export system, the type III secretion system (T3SS), capable of transporting effector proteins across three membranes: the two membranes of the bacterial cell envelope and the cell membrane of the targeted cell (7). The T3SS has a needle-like shape that allows effectors to be exported across both bacterial membranes and into the cytoplasm of the targeted cell without being exposed to the extracellular milieu (13). Strains possessing T3SS include *Salmonella*, *Shigella*, and *Yersinia* species which are capable of controlling trafficking events in the phagosome, the cellular compartment formed by the fusion of the cell membrane around the invading pathogen.

The bacterial type IV secretion system (T4SS) is a membrane-associated transporter used by several Gram-positive and Gram-negative bacteria. This transporter is related to bacterial conjugation because it can transfer genetic material to other bacterial cells by horizontal gene transfer. The T4SS exports virulence factors into mammalian cells (5, 6). Several human pathogens such as *Legionella*, *Brucella*, and *Coxiella* species possess the T4SS and achieve intracellular survival by inhabiting vacuoles from the endocytic pathway (6).

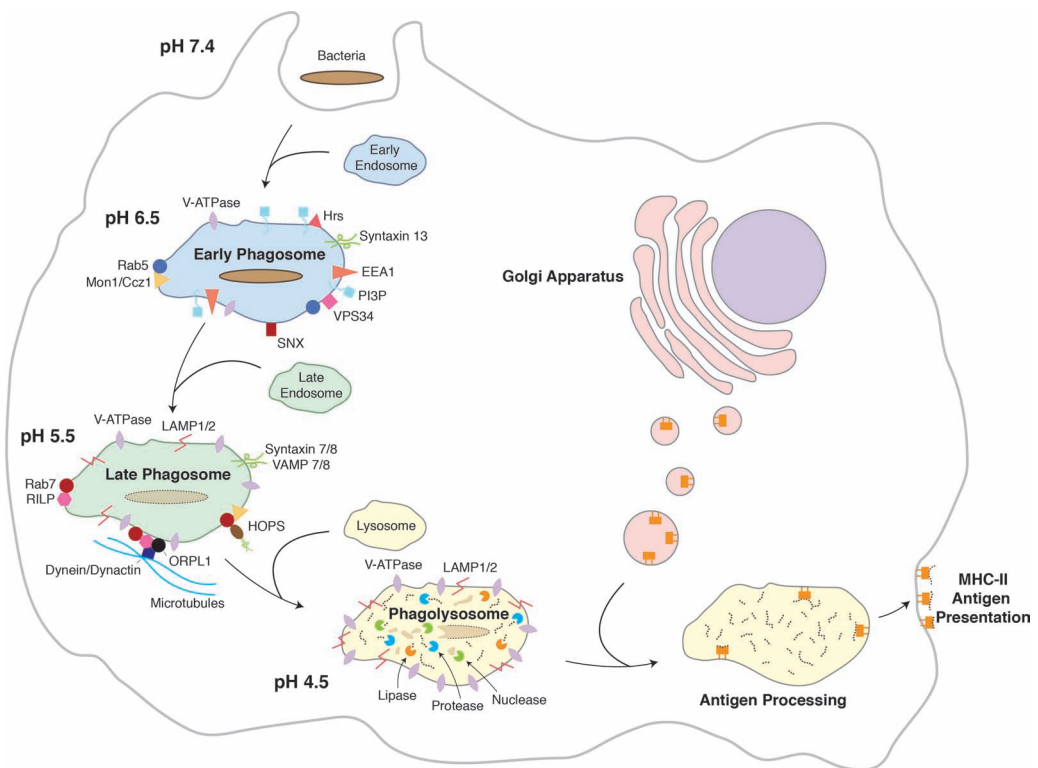
The type VII secretion system (T7SS) distantly resembles T4SS (14) and is found in certain species of Gram-positive bacteria. The particularity of this specialized secretion system is its ability to ensure transport of virulence factors across the complex cell wall of acid-fast *Mycobacterium* species (15). Mycobacteria have a distinctive cell envelope structure that is characterized by an exceptionally hydrophobic, impermeable and thick layer, termed the mycomembrane. The unique features of the membrane are due to the presence of mycolic acids, which are large branched-chain fatty acids. In mycobacteria, the T7SS includes the ESX 1 to 5 secretion systems, some of which are essential for virulence and take active roles in macrophage escape and cell-to-cell spread

(16, 17). Despite the absence of the mycomembrane typical of *Mycobacterium* species, Gram-positive bacteria such as *Streptomyces* and *Listeria* species also possess a T7SS (10).

## PHAGOSOME MATURATION

Upon engulfment by a phagocyte, microorganisms are trapped in an organelle derived from the plasma membrane, termed the phagosome. Phagosomes acquire microbicidal features that enable them to kill and digest engulfed microbes through a process known as phagosome maturation. Phagosome maturation includes a variety of fusion and fission events with compartments of the endocytic

pathway whereby the contents of the phagosome are gradually delivered to lysosomes for degradation (18). Phagosome maturation radically alters the composition of the phagosome, converting it into a potent microbicidal organelle (18). As illustrated in Fig. 1, phagosomes containing foreign particles, such as invading microorganisms, interact with the endosomal pathway, allowing for the exchange of solute materials and membrane components between phagosomes and endosomes. Sequential interactions with endosomal compartments and lysosomes yield mature phagolysosomes that are markedly acidic due to the reduction in phagosomal pH resulting from the acquisition of vacuolar H<sup>+</sup>-ATPase (V-ATPase) pumps (18). These



**FIGURE 1** Stages of phagosome maturation. During phagocytosis, the phagosome undergoes a series of fusion and fission events with vesicles of the endocytic pathway, culminating in the formation of the phagolysosome. Maturation of the phagosome involves gradual decrease in pH and acquisition of antimicrobial properties, leading to the digestion of the invader and presentation of antigens on the surface of the phagocyte by MHC-II molecules.

fusion events modify the function of the phagosome to reflect the content of the lysosome, which is highly oxidative and enriched with hydrolytic enzymes (18). Invading microorganisms are ultimately degraded, and their peptides are presented on the surface of the phagocyte to initiate an adaptive immune response (19).

Although phagocytosis normally results in the eradication of microorganisms, some pathogens have developed strategies to interfere with phagosome maturation and use phagocytes as niches for survival and growth. Different stages of phagosome maturation can be targeted by different microorganisms: the fusion of the phagosome with early and late endosomes, the fusion with lysosomes, the acidification of the phagosome, the redirection of the phagosome to a nondigestive route, etc. These events create an alcove suited for bacterial replication.

While the list of effector macromolecules secreted by pathogens suggested to cross-talk with host proteins or specific host pathways is growing, the precise mechanisms of communication that allow pathogens to interfere with defined host proteins (e.g., signaling and metabolic proteins), and to survive and replicate within the hostile environment of the host, are still very limited and poorly understood. In this chapter, the current knowledge of a subset of bacterial pathogen effectors involved in altering the phagosome to circumvent pathogen destruction is summarized (Table 1). Each step of the phagosome maturation process is examined individually, and the effectors of selected pathogens involved in interfering with this process are described. In particular, effectors secreted by the Gram-negative bacteria are characterized: *Legionella*, which causes the acute lung disease Legionnaires' disease (*Legionella pneumophila*) (20), *Salmonella*, responsible for the localized small intestine disease salmonellosis (*Salmonella enterica*) and systemic disease typhoid fever (*Salmonella typhi*), *Shigella*, the causative agent of the small intestine disease shigellosis (*Shigella dysen-*

*teriae*, *Shigella flexneri*, *Shigella sonnei*), and *Yersinia*, the agent responsible for the black death, or bubonic/pneumonic plague (*Yersinia pestis*). Moreover, effectors secreted by the Gram-positive bacterium are enumerated: *Listeria*, responsible for listeriosis, an infection of the central nervous system or the small intestine (*Listeria monocytogenes*), and the acid-fast actinomycete *Mycobacterium*, the causative agent of diseases such as tuberculosis (*Mycobacterium tuberculosis*) (21). Identifying these effectors and their modes of action is essential to understanding the pathogenesis of diseases and how pathogens manipulate the defense mechanisms of the host to their advantage.

### Targeting Phagosome-Endosome Fusion

As illustrated in Fig. 1 and described in detail in Fig. 2, the metamorphosis of the phagosomal membrane occurs after engulfment of the invader. Changes begin immediately and are coordinated by Rab GTPases, a family of molecular switches, which alternate between an active (GTP-bound) and an inactive (GDP-bound) state. Their activity is controlled by Rab GDP dissociation inhibitors, guanine nucleotide exchange factors (GEFs), and GTPase-activating proteins (22). Once activated, Rab molecules regulate host vesicular and membrane transport processes by modulating membrane structure and function (22).

Upon phagosome biogenesis, the pathogen-containing phagosome recruits the early endosomal marker, Rab5, which coordinates traffic between the phagosome and early endosomes (18). Rab5 recruits effector molecules such as the class III phosphatidylinositol 3-kinase vacuolar protein sorting 34 (VPS34) (23). VPS34 catalyzes the production of the lipid regulator phosphatidylinositol 3-phosphate (PI3P) from phosphatidylinositol (PI) (23). PI3P, in turn, is a phagosomal membrane tag that signals phagosomes to progress down the phagolysosome biogenesis pathway (24). To achieve fusion of the early phagosome with

endosomes, PI3P affects the localization and function of specific proteins involved in membrane trafficking, endosomal protein sorting, and multisubunit enzyme assembly at the membrane (25). These proteins include early endosome antigen 1 (EEA1), p40 subunit of the NADPH oxidase, and hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) (24, 26, 27). EEA1 facilitates docking and fusion of the early phagosome with early organelles of the endocytic pathway via interaction with syntaxin 13, a soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein (28). SNAREs assemble downstream of tethering molecules and drive fusion of membranes (29).

Following the fusion with early endosomes, the phagosome acquires the late endosomal marker, Rab7, which prompts fusion of the early phagosome with late endosomes and the acquisition of additional factors contributing to microbicidal functions, such as the integral membrane proteins lysosome-associated membrane proteins 1/2 (LAMP1 and LAMP2) and V-ATPases (18). The presence of additional V-ATPase pumps in the late phagosome further acidifies the organelle (luminal pH ~ 5.5).

Pathogenic mycobacterial species inhibit fusion of the phagosome with endosomes. The *M. tuberculosis* cell wall glycolipid mannosylated lipoarabinomannan (ManLAM) is secreted into the host cytoplasm (30) and inhibits intracellular calcium ( $\text{Ca}^{2+}$ ) levels, resulting in the disruption of phagosome maturation (31).  $\text{Ca}^{2+}$  is an essential cell signaling molecule, and increases in intracellular  $\text{Ca}^{2+}$  following infection lead to the accumulation of the small GTPase Rab5 in the phagosomal membrane. As seen in Fig. 2, upon secretion of ManLAM, intracellular  $\text{Ca}^{2+}$  is reduced and Rab5 only partially localizes to the phagosomal membrane. The recruitment of Rab5's effector VPS34 to the phagosomal membrane is consequently hindered. Since PI3P is partly excluded from the phagosome, PI3P-binding proteins, such as EEA1, only accumulate in small amounts (32). Thus, ManLAM reduces the fusion of

the phagosome with early endosomes and the delivery of the endosomal cargo between them (33).

Partial PI3P exclusion mediated by ManLAM is not sufficient to cause complete inhibition of phagosome and endosome fusion. *M. tuberculosis* secretes an acid phosphatase termed secreted acid phosphatase of *M. tuberculosis* (SapM), which hydrolyzes host PI3P into PI (32). Dephosphorylation of PI3P into PI prevents PI3P accumulation at the phagosomal membrane, impeding EEA1 recruitment and fusion with endosomes (32). The means by which ManLAM and SapM are able to cross the phagosomal membrane and gain access to the cytoplasmic face of the phagosome, where they prevent intracellular  $\text{Ca}^{2+}$  levels from rising and hydrolyze PI3P, remains a conundrum (32).

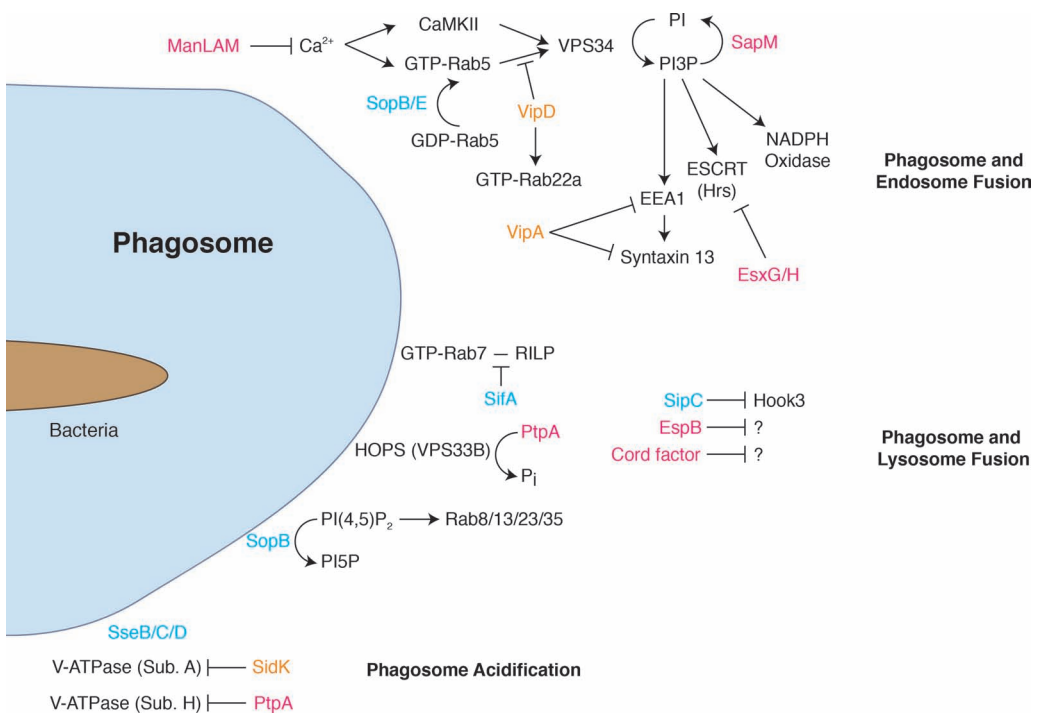
*Legionella* species are also able to inhibit fusion of the phagosome with endosomes. The *L. pneumophila* effectors VipD and VipA interfere with early and late endosomal transport, respectively. VipD tightly binds to the GTP-bound form of the early endosomal markers Rab5 and Rab22a, limiting interactions with downstream effector molecules and inhibiting endocytic trafficking (34). VipA possesses a coiled-coil region and is suspected of interacting with host proteins that also contain coiled-coil regions such as SNAREs and EEA1 (Fig. 2) (35).

*Salmonella* species, such as *S. enterica*, promote fusion of the phagosome with endosomes but inhibit fusion with lysosomes. Interestingly, *S. enterica* promotes fusion of the phagosome with endosomes by secreting the effectors *Salmonella* outer protein B/E (SopB and SopE), which recruit the small GTPase Rab5 to the phagosomal membrane and activate it by subverting inactive GDP-bound Rab5 for active GTP-bound Rab5 (Fig. 2) (36–38). In addition, studies have shown that SopE promotes retaining Rab5 on the phagosome by stimulating GDP to GTP nucleotide exchange of Rho GTPases (39). Retaining active Rab5 on the phagosomal membrane thus promotes fusion of the

**TABLE 1** Host physiological events and substrates targeted by effectors secreted by *Legionella*, *Listeria*, *Mycobacterium*, *Salmonella*, *Shigella*, and *Yersinia* species

Biological event targeted	Pathogen	Bacterial effector	Host target	Refs.	
Endosomal trafficking	<i>Mycobacterium tuberculosis</i>	ManLAM	Reduces intracellular Ca <sup>2+</sup> concentration	31	
		SapM	Hydrolyzes PI3P into PI	32	
	<i>Legionella pneumophila</i>	VipD	Interacts with GTP-Rab5 and GTP-Rab22a	34	
		VipA	Interacts with EEA1 and SNAREs	35	
	<i>Salmonella enterica</i>	SopB	Recruits Rab5	36	
		SopE	Recruits and activates Rab5	37, 38	
Phagosome and lysosome fusion	<i>S. enterica</i>	SifA	Uncouples Rab7 from RILP	45	
		SipC	Inactivates Hook3	46	
		SopB	Hydrolyzes PI(4,5)P <sub>2</sub> into PI5P, reducing the recruitment of Rab8, Rab13, Rab23, and Rab35	48	
		PtpA	Dephosphorylates VPS33B	49	
	<i>M. tuberculosis</i>	EsxG/H	Form a complex that targets Hrs, a component of the ESCRT machinery.	51	
		EspB	Inhibits phagolysosome fusion when cosecreted with ESAT-6 and CFP-10	53	
		Cord factor	Creates a steric block to fusion and/or increases the hydration force between two phospholipid bilayers	56	
	Phagosome acidification	<i>Yersinia pestis</i>	Unknown	Resides and replicates in a phagolysosome-like vacuole	63, 64
		<i>M. tuberculosis</i>	PtpA	Binds subunit H of V-ATPase and prevents assembly of the proton pump	66
		<i>L. pneumophila</i>	SidK	Binds subunit A of V-ATPase and inhibits ATP hydrolysis and proton translocation	67
<i>Yersinia pseudotuberculosis</i>		Unknown	Decreases the activity of the V-ATPase pump	59	
<i>S. enterica</i>		SseB/C/D	Forms a complex that helps in the translocation of T3SS effectors across the bacterial membrane	69	
Cytoskeleton remodeling	<i>S. enterica</i>	SipA	Catalyzes actin polymerization and bundling of actin filaments. Stabilizes SifA via its actin modification effects	71, 74	
		SipC	Bundles and nucleates actin filaments	73	
		SseI	Interacts with filamin A and promotes cross-linking of F-actin by filamin A	72	
		SspH2	Interacts with filamin A and promotes cross-linking of F-actin by filamin A	72	
		SpvB	Depolymerizes and disrupts the actin cytoskeleton by modifying G-actin	75	
		SopB	Indirectly recruits SNX3, which forms tubules for the movement of the phagosome to the perinuclear region	76	
		PipB2	Interacts with kinesin-1 and forms Sifs	81	
		SifA	Interacts with SKIP, forms Sifs, promotes phagosomal tubulation, and uncouples Rab7 from RILP	45, 76, 81, 84	
		SseJ	Interacts with SKIP and GTPase RhoA and promotes phagosomal tubulation	84	

		SseF/G	Participates in the dynein-mediated movement of the phagosome along microtubules; serves as a scaffold for Sif formation	87, 88
	<i>L. pneumophila</i>	VipA	Binds actin and enhances its polymerization	89
	<i>Mycobacterium marinum</i>	Unknown	Recruits WASp and induces the formation of actin tails	90
	<i>Listeria monocytogenes</i>	ActA	Mimics WASp to induce actin polymerization	92
	<i>Shigella flexneri</i>	IcsA	Recruits neural WASp to induce actin polymerization	93
Vacuolar membrane lysis	<i>M. marinum</i>	Unknown	Escapes from the phagosome	90
	<i>S. flexneri</i>	IpgD	Hydrolyzes PI(4,5)P <sub>2</sub> into PI5P, which recruits EGFR and Rab11-positive vacuoles	94–96
		IpaB	Forms pores in the vacuole membrane	97, 98
		IpaC	Disrupts the integrity of the phospholipid bilayer of vesicles	97
		IpaH7.8	Promotes bacterial phagosome escape	100
	<i>L. monocytogenes</i>	LLO	Forms pores in the vacuole membrane	101
		PI/PC-PLC	Causes the breakdown of the vacuole membrane	102
Phagosomal Membrane Remodeling	<i>L. pneumophila</i>	SidF	Hydrolyzes PI(3,4)P <sub>2</sub> and PI(3,4,5)P <sub>3</sub> into PI4P and PI(4,5)P <sub>2</sub>	105
		LidA	Recruits Rab1	108
		DrrA/SidM	Recruits and converts inactive GDP-Rab1 into active GTP-Rab1 and maintains it on the phagosomal membrane; AMPylates Rab1	108, 111, 112
		LepB	Converts active GTP-Rab1 into inactive GDP-Rab1 and releases it from the phagosomal membrane	114
		SidD	DeAMPylates Rab1	115
		AnkX	Catalyzes the attachment of a phosphocholine moiety (phosphocholination) to GTPases Rab1 and Rab35	116, 117
		Lem3	Reverses post-translational modification (dephosphocholination) on Rab1	119
		RalF	Recruits ARF1 to the membrane and activates it	120
		SidJ	Modulates host processes to redirect the recruitment of ER-derived vesicles to the phagosome	123
		SidP	Hydrolyzes PI3P and PI(3,5)P <sub>2</sub> promoting the evasion of the endocytic pathway by the phagosome	124
		SidC	Acts as a tethering factor for the recruitment of ER-derived vesicles to the phagosome	122
		SetA	Binds PI3P and impairs vesicular trafficking via its glycosyltransferase activity	125



**FIGURE 2** Microbial effectors interfering with intracellular trafficking and acidification events. Orange proteins represent *Legionella* virulence factors; pink, *Mycobacterium* virulence factors; and blue, *Salmonella* virulence factors.

phagosome with early endosomes via the recruitment of VPS34, as previously described, and explains the relatively large membrane-bound vesicle size in which *Salmonella* resides (40). Moreover, previous studies have demonstrated that phagosomes containing pathogenic *Listeria* and *Mycobacterium* species are enriched in Rab5, thereby inhibiting their transport to lysosomes (41, 42). Thus, the prolonged period of time spent in the mildly acidic early phagosome may account for blocking transport of the *Salmonella*-containing phagosome to lysosomes and *Salmonella*'s survival.

### Targeting Phagosome-Lysosome Fusion

The mature phagosome is the ultimate microbicidal and degradative organelle. To complete phagosome maturation, the small GTPase Rab7 recruits Rab-interacting lyso-

somal protein (RILP) and oxysterol-binding protein-related protein 1L (ORP1L) to the late phagosome (Fig. 1) (18). RILP is a dynein adaptor, and ORP1L regulates the binding of RILP to dynein. Together, RILP and ORP1L interact with the dynein-dynactin molecular motor and coordinate microtubule-dependent vesicular trafficking of the late phagosome to the microtubule organizing center, a perinuclear region near the Golgi apparatus, where fusion with lysosomes occurs (18). Rab7 also associates with the homotypic fusion and vacuole protein sorting (HOPS) complex, a large multimeric tethering factor essential for vesicle fusion (43). The HOPS complex is composed of VPS11, VPS16, VPS18, VPS33B, VPS39, and VPS41 (18). The HOPS complex is needed during the tethering and docking stages of vesicle fusion between the phagosome and the lysosomes (44), where it regulates the assembly of SNARE molecules



such as syntaxin 7 and vesicle-associated membrane protein 7 at the phagosomal membrane (18). Phagolysosome fusion permits the exchange of cytosolic contents such as hydrolases (nucleases, lipases, proteases, etc.), and additional LAMP1/2 proteins and V-ATPase pumps (Fig. 1) (18). The phagolysosome possesses a strong acidic luminal (pH ~ 4.5) which, along with the action of hydrolytic enzymes and oxidants, contributes to the degradation of microorganisms. Lastly, the phagolysosome subsequently fuses with Golgi vesicles carrying major histocompatibility class II molecules for antigen processing and presentation (18).

Late endocytic and lysosomal markers are often the targets of choice for pathogens because inhibition or inactivation of these targets guards bacteria from exposure to microbicidal compounds. As illustrated in Fig. 2, the *Salmonella* effectors *Salmonella*-induced filaments A (SifA) and *Salmonella* invasion protein C (SipC) block phagolysosome fusion by uncoupling Rab7 from RILP (45) and inactivating Hook3, a mammalian protein implicated in cellular trafficking (46), respectively. In addition to recruiting the small GTPase Rab5 to the phagosome, the SopB effector acts as a PI phosphatase. PI lipids are important regulators of cellular processes such as cell signaling, cytoskeleton remodeling, and membrane trafficking (47). SopB alters the PI composition of the phagosomal membrane by hydrolyzing phosphatidylinositol 4,5-bisphosphate (PI[4,5]P<sub>2</sub>) into phosphatidylinositol 5-phosphate (PI5P), reducing the recruitment of Rab8, Rab13, Rab23, and Rab35 while preventing phagolysosome fusion (48).

Pathogenic *Mycobacterium* species interfere with phagolysosome fusion by secreting effectors which have been shown to interact with the host proteins PtpA, EsxG, EsxH, EspB, and cord factor (Fig. 2). The low-molecular-weight tyrosine phosphatase, protein tyrosine phosphatase A (PtpA), translocates to the host cytosol, where it dephosphorylates VPS33B (49). As a member of the HOPS complex,

VPS33B plays a key role in the regulation of vesicle trafficking and membrane fusion in the endocytic pathway (50). Dephosphorylation of VPS33B by PtpA disrupts the assembly of the HOPS complex and translates directly into phagosome maturation arrest and avoidance of proteolytic degradation (49). EsxG and EsxH are secreted by mycobacteria and target the component of the host endosomal sorting complexes required for transport (ESCRT) machinery, Hrs (51). The ESCRT machinery directs cargo destined for degradation to lysosomes (52). However, the combined action of EsxG and EsxH disrupts ESCRT function and impairs phagolysosome fusion, preventing delivery of *M. tuberculosis* to the lysosome (51). In addition, the EspB effector, when combined with other mycobacterial antigens, increases phagosome maturation inhibition (53). Indeed, cosecretion of EspB with the 6-kDa early secretory antigenic target (ESAT-6) and 10-kDa culture filtrate protein (CFP-10) enhances inhibition of phagosome maturation and promotes survival of the pathogen (53). However, the mechanism of action which allows EspB to prevent phagolysosome fusion and its target remains unknown.

Finally, cord factor is the most abundant glycolipid found in the mycobacterial cell wall, and it interferes with phagolysosome fusion (54). Cord factor consists of the disaccharide trehalose covalently bound to two mycolic acid residues, which in turn are anchored to the bacterial membrane by the hydrophobic component. Such molecules have been observed to confer fusion inhibition of phospholipid bilayers (55). In agreement with this, cord factor is thought to act as a barrier and prevent the fusion of phospholipid vesicles such as phagosomes and lysosomes. The mechanism by which cord factor is transferred from the bacterial cell to the phagosomal membrane, and how it blocks phagolysosome fusion, remains unclear. However, phagolysosome fusion inhibition is believed to be due to cord factor creating a steric block to fusion and/or increasing the hydration force (56).

*Yersinia*'s primary niche for replication is extracellular. Thus, *Yersinia* synthesizes a large number of effectors that block phagocytosis and promote extracellular growth. In spite of this antiphagocytotic effort, a significant amount of its microbial population is engulfed by macrophages (57). The ability of *Yersinia* to replicate in macrophages remains a disputed issue despite several studies supporting this claim (58–60). Unlike other intracellular pathogens which have developed multiple strategies to inhibit phagosome fusion (61, 62), certain strains of *Yersinia* have been reported to reside within the phagolysosome (63). These results indicate that, as observed in *Salmonella* infection, the *Yersinia*-containing phagosome acquires lysosomal markers before being excluded from the lysosomal pathway (64). Transient interactions with lysosomes may be mandatory for remodeling the phagosome into a replication-permissive vacuole. Over the years, considerable attention has been given to how *Yersinia* manipulates the functions of macrophages from the outside, but little is known about the modes of action behind the intracellular subversion of macrophage function.

### Inhibition of Phagosomal Acidification

The impressive destructive capacity of the phagolysosome is attributed to the concerted effort of molecules, such as hydrolytic enzymes and oxidants, plus the acidification of the phagosome. The acidification of the phagosome is generated by the V-ATPase pump, a protein complex that controls phagosome acidification by transporting protons across membranes (65). The acidification of the phagolysosome serves several purposes: it restricts microbial growth, it activates lysosomal hydrolases whose activity is optimal at low pH, and intraphagosomal protons are used to produce reactive oxygen species which are important antimicrobial ammunition for phagocytes (18).

Given the importance of phagosome acidification, it is not surprising that several

pathogens have developed strategies to block phagosome acidification by targeting the proton pump, allowing them to remain in a relatively neutral pH where they can survive. As seen in Fig. 2, the *M. tuberculosis* secreted phosphatase PtpA directly interferes with phagosome acidification by blocking the assembly of the macrophage's V-ATPase pump (66). Specifically, PtpA binds to subunit H of the pump and excludes the pump from the phagosomal membrane, resulting in diminished phagosome acidification (66). In a similar manner, the *L. pneumophila* protein SidK interacts with subunit A of the V-ATPase pump and inhibits ATP hydrolysis and proton translocation, resulting in a fairly neutral pH inside the phagosome (67).

*Yersinia* has also been shown to prevent acidification of phagolysosomes. Unlike pathogenic mycobacteria, which inhibit acidification of the phagosome by excluding the proton pump from the phagosomal membrane, *Y. pseudotuberculosis* attenuates the activity of the V-ATPase pump (59). To date, however, no *Yersinia* effectors inhibiting phagosomal acidification have been identified.

Contrary to *Mycobacterium*, *Legionella*, or *Yersinia* species, *Salmonella* species do not interfere with phagosome acidification. Instead, they adapt to the lower phagosomal pH. *Salmonella*'s adaptive response involves activation of acid tolerance genes which help the bacterium cope with the acidic pH (68). Upon exposure to the acidic environment, *Salmonella* secretes secreted system effector B/C/D (SseB, SseC, and SseD) to its surface, where they form a complex and participate in the translocation of T3SS effectors (see below) across the bacterial membrane (69).

### SURVIVAL STRATEGIES OF INTRACELLULAR PATHOGENS BEYOND PHAGOSOME MATURATION ARREST

To avoid prolonged exposure to the harsh environment of the phagolysosome, intracellular pathogens have developed alternative

survival strategies. In addition to prevention of phagosome maturation or acidification, some pathogens, such as *Salmonella* and *Chlamydia*, relocalize the phagosome outside of the endocytic pathway where they can replicate. Others, exemplified by *Shigella* and *Listeria*, escape the phagosome before fusion with lysosomes and replicate in the host cytoplasm. Alternatively, *Legionella* forces the remodeling of the phagosomal membrane into a replicative-permissive vacuole (70). These numerous strategies suggest several ways to circumvent the killing capacity of the phagosomal pathway.

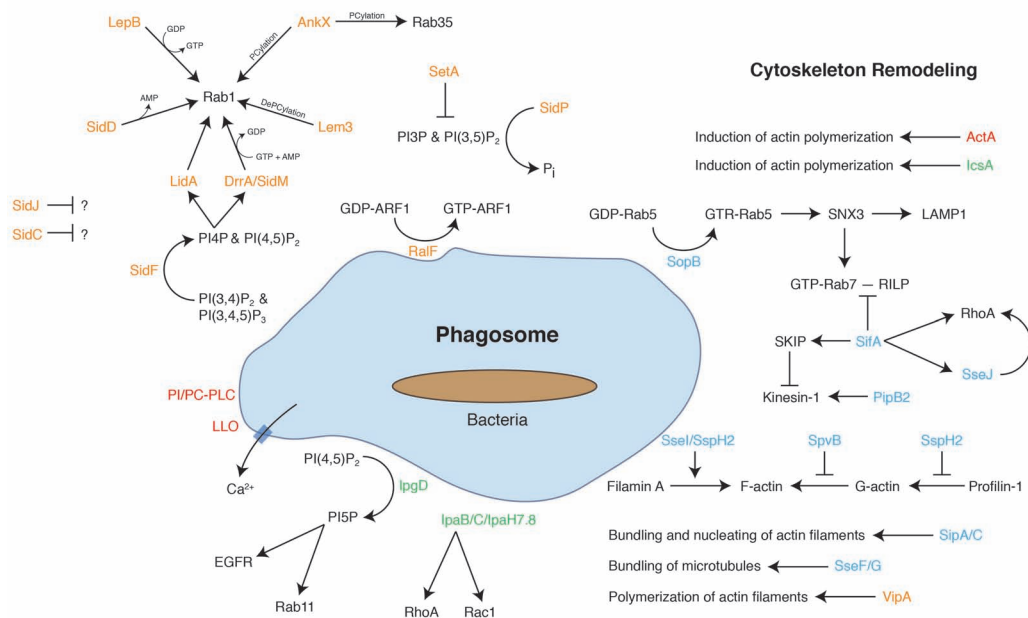
### Cytoskeleton Remodeling

After blocking the digestive endocytic pathway, certain pathogens require the localization of the phagosome to areas of the cell

where acquisition of nutrients or membrane components from organelles occurs. To establish a replication-permissive vacuole, pathogens manipulate actin polymerization and form an intermediate filament network around the phagosome, allowing for the rerouting of the pathogen-containing phagosome.

To replicate, *Salmonella* must localize to the microtubule organizing center near the Golgi apparatus. This migration is ensured by the formation of an actin network around the phagosome (71). As illustrated in Fig. 3, the effectors *Salmonella* invasion protein (Sip) AC, secretion system effector I (SseI), *Salmonella*-secreted protein H2 (SspH2), and *Salmonella* plasmid virulence protein B (SpvB) take active roles in the formation of this network (72). SipA and SipC cause actin condensation and cytoskeletal rearrangements by bundling and nucleating actin

#### Phagosomal Membrane Remodeling



#### Vacuolar Membrane Lysis

**FIGURE 3** Cytoskeleton remodeling, vacuolar membrane lysis, and phagosomal membrane remodeling by microbial pathogens. Orange proteins represent *Legionella* virulence factors; red, *Listeria* virulence factors; blue, *Salmonella* virulence factors; and green, *Shigella* virulence factors.

filaments (71, 73, 74), while both SseI and SspH2 interact with the host actin cross-linking protein filamin A for cross-linking F-actin (72). The cross-linking of F-actin is important for remodeling the cytoskeleton for modulation of cell shape and motility and for vesicle and organelle movement. Moreover, SspH2 interacts with profilin-1, another actin-binding protein, and thus prevents the interaction of profilin-1 with G-actin and alters the rate of actin polymerization (72). The redistribution of the phagosome away from the perinuclear region occurs by the depolymerization and disruption of the actin cytoskeleton of the host cell. Termination of actin polymerization is carried by the SpvB effector, which post-translationally modifies G-actin monomers, preventing their polymerization into F-actin filaments (75).

The proper positioning of the *Salmonella*-containing phagosome near the Golgi apparatus is dependent on the formation of tubules from the phagosome. The SopB effector is required early in infection to recruit Rab5 to the early phagosome (36). Rab5 subsequently recruits sorting nexin 3 (SNX3), an important regulator of membrane trafficking, which contributes to the recruitment of Rab7 and LAMP1 to the phagosome (Fig. 3). SNX3 also promotes the formation of tubules and the movement of the phagosome (76).

Upon localization of the phagosome near the Golgi apparatus, phagosome maturation stops, replication of the pathogen is initiated, and specialized filamentous membrane structures named *Salmonella*-induced filaments (Sifs) form. Sifs are derived from late endosomes, because they contain late endocytic markers such as LAMP1 and V-ATPase (77). They extend from the surface of the phagosome along microtubules to the cell periphery, where they recruit host Rab9 and Rab11 which regulate fusion with Golgi-derived vesicles (78, 79). Sifs also contribute to the localization of endocytic compartments to the cell periphery for nutrient acquisition, the movement of bacteria from cell to cell, and the enlargement of the phagosome to

accommodate growing numbers of replicating bacteria (80).

The formation of Sifs is principally dependent on two effectors, PipB2 and SifA (80, 81), and to a lesser extent on SseJ, SseF, and SseG (Fig. 3) (82). On the one hand, PipB2 promotes the outward, or anterograde, movement of the phagosome by recruiting host kinesin-1 to the phagosomal membrane (81). Kinesin-1 is a microtubule motor complex that transports intracellular cargo to the cell periphery (83). On the other hand, SifA, necessary for the stability of the phagosome, promotes the movement of the phagosome toward the perinuclear region (80). In contrast to PipB2, SifA downregulates the recruitment of kinesin-1 by interacting with the host protein SifA kinesin-interacting protein (SKIP) (81). SKIP binds kinesin-1 and regulates kinesin-1 levels at the phagosomal membrane (84). The formation of Sifs requires a balance between the activities of PipB2 and SifA, and this balance is influenced by the actin-binding protein SipA, which stabilizes SifA via its actin-modulatory effects (74). Thus, the counteracting functions of PipB2 and SifA suggest that opposing as well as complementary activities of *Salmonella* effectors are required for Sif formation. Moreover, in a parallel pathway, the SifA-mediated uncoupling of Rab7 from RILP is also believed to facilitate the extension of tubules from the phagosome as SifA binding to Rab7 displaces RILP/dynein-dynactin from Sifs (45).

In cooperation with SifA, the effector SseJ also contributes to Sif formation by controlling the dynamics of the phagosome. Upon recruitment by SifA, SKIP and the small GTPase RhoA form a complex with SseJ which promotes the induction of tubular filaments from the phagosomal membrane (85). The exact mechanism of tubulation induction is currently unknown. The small GTPase RhoA, known to participate in the regulation of microtubule dynamics, regulates kinesin-1 binding to the microtubule and, therefore, kinesin-1-mediated transport

(86). SseF and SseG participate in the dynein-mediated movement of the phagosome along microtubules. Indeed, SseF and SseG colocalize with microtubules, induce their bundling, which serves as a scaffold for Sif formation, and control the positioning of the phagosome by modulating the activity of dynein on the phagosome (87). In addition to their role in Sif formation, SseF and SseG also promote the aggregation of endosomal vesicles into tubules and recruit Golgi-derived vesicles to the phagosome, indicating that interactions with the secretory pathway are required for intracellular replication (88).

*Legionella* also secretes effectors that interfere with host cell organelle trafficking pathways. *In vitro* studies have shown that the effector VipA colocalizes with early endosomes and host cell actin filaments and causes a direct enhancement of microfilament polymerization (89). This helps isolate the phagosome from the endocytic pathway and enables the pathogen to escape degradation.

Lastly, cytoskeleton remodeling has also been observed in the *Mycobacterium* species *Mycobacterium marinum*, a pathogen of fish and frogs. *M. marinum* escapes the phagosome and is free in the host cytoplasm, where it manipulates the actin filament network of the host to induce the formation of actin tails. The use of actin-based motility propels the pathogen through the cell cytoplasm to the cell periphery or into neighboring cells. This behavior has only been observed in this specific *Mycobacterium* species (90). In a similar fashion, *Listeria* and *Shigella* also utilize the host actin assembly machinery to move within the host and spread between host cells (91). During normal actin remodeling in host cells, members of the Wiskott-Aldrich syndrome protein (WASp) family activate the actin-related protein 2/3 (Arp2/3) complex. *Listeria* and *Shigella* have both developed mechanisms to induce actin polymerization by activating the Arp2/3 complex. As shown in Fig. 3, *Listeria* releases ActA (92), which mimics WASp, and *Shigella*

secretes IcsA (93), which recruits neural WASp to the bacterial surface. Both host proteins activate the Arp2/3 complex (91). The mechanism by which *M. marinum* induces actin polymerization remains incomplete, but studies have shown that *M. marinum* recruits WASp to its surface, and its mode of action shares more similarities to that of *Shigella* than *Listeria* (90).

### Lysis of the Vacuolar Membrane

*Shigella*, *Listeria*, and *M. marinum* have all developed mechanisms enabling them to lyse the membrane of the vacuole in which they reside, permitting their escape into the host cytoplasm. The mechanism by which *M. marinum* escapes the phagosome remains unknown. During infection, *Shigella* secretes the PI phosphatase IpgD, which changes the lipid composition of the early endosomal membrane (Fig. 3). IpgD dephosphorylates PI(4,5)P<sub>2</sub> to produce PI5P (94). PI5P recruits epidermal growth factor receptor (EGFR) to the membrane, and prolonged signaling via EGFR slows down phagosome maturation and impairs lysosomal degradation (95). In addition, PI5P also recruits Rab11-positive vacuoles to the phagosome, aiding in the process of vacuolar rupture (96). During the late phagosomal stage *Shigella* releases IpaB and IpaC, two virulence factors that facilitate membrane lysis. In particular, IpaB forms pores within membranes, while IpaC disrupts membrane integrity (97, 98). The specific mechanism by which pores are formed remains to be discovered, but it is suggested to involve host signaling as the small GTPases RhoA and Rac1 are recruited to the lysing vacuoles (99). In addition to IpaB and IpaC, IpaH7.8 has been suggested to promote bacterial phagosomal escape, but its participation in this process is controversial (100). As previously described, once the pathogen escapes into the cytoplasm, it releases effectors that cause actin polymerization reorganization to facilitate its intracellular motility inside the host cell.

*Listeria* also perforates the phagosomal membrane and escapes into the cytoplasm in a process mediated by the secretion of listeriolysin O (101). In addition, *Listeria* releases the phospholipases phosphatidylinositol phospholipase C and phosphatidylcholine phospholipase C, which also contribute to phagosomal membrane breakdown (Fig. 3) (102).

### Membrane Remodeling of the *Legionella*-Containing Phagosome

To bypass the conventional endocytic maturation route, *Legionella* has developed the ability to modify the composition of the phagosomal membrane into a replicative-permissive compartment analogous to endoplasmic reticulum (ER)-derived vesicles (103). This remodeling process is unique to *Legionella* and occurs in sequential steps soon after phagocytosis. First, the bacterium intercepts early secretory vesicles from the ER and associates them with the phagosome. These ER vesicles then fuse to each other to form a large structure surrounding the phagosome (103, 104). The second stage involves the elimination of the phagosomal membrane allowing *Legionella* to replicate in this new ER-like compartment (103).

To allow fusion with ER-derived vesicles, the *Legionella*-harboring phagosome first remodels its own membrane via a process that involves PI metabolism (103). *Legionella* secretes SidF, a phosphoinositide 3-phosphatase, which hydrolyzes PI(3,4)P<sub>2</sub> and PI(3,4,5)P<sub>3</sub>, the two PI species generated on the phagosome upon phagocytosis, into PI4P and PI(4,5)P<sub>2</sub> (Fig. 3) (105). As a result, the lipid composition of the phagosome resembles that of the Golgi apparatus, an appealing site for ER-derived vesicles.

The SidF-mediated conversion of the phagosome into a PI4P enriched organelle anchors LidA and DrrA/SidM, two effectors that promote the recruitment of ER-derived vesicles and fusion with the phagosome (106, 107). As illustrated in Fig. 3, LidA and DrrA/

SidM recruit early secretory vesicles to the phagosome via interaction with the host GTPase Rab1, which plays a distinct role in the maturation of phagosomes (108). Rab1 is necessary for the fusion of ER-derived vesicles with the Golgi apparatus (109), conferring an ER-like membrane to the phagosomes (110). The DrrA/SidM effector performs two covalent modifications on Rab1; its GEF domain recruits Rab1 to the phagosome and converts inactive GDP-bound Rab1 into active GTP-bound Rab1 (111), and its nucleotidyltransferase domain transfers an adenosine monophosphate (AMPylation) to a tyrosine residue on Rab1, which contributes to maintaining it on the phagosomal membrane and disrupting vesicular transport (112). Active Rab1 then recruits host-tethering factors to the phagosomal membrane and facilitates membrane fusion with ER-derived vesicles (110). Rab1 activity is also regulated by LepB, a GTPase-activating protein (113). However, unlike DrrA/SidM, LepB regulates the removal of Rab1 from membranes by binding to GTP-bound Rab1 and promoting GTP hydrolysis, which results in inactivated Rab1 (114). SidD also functions as an antagonist of DrrA/SidM, contributing to its release from the membrane (Fig. 3) (115).

The effector ankyrin repeat-containing protein X (AnkX), a phosphorylcholine transferase, also modifies the small GTPase Rab1. It does so by catalyzing the attachment of a phosphocholine moiety (PCylation) to a serine residue of Rab1 (116). This covalent modification interferes with the GTPase activity of Rab1, preventing it from interacting with cellular effectors and impeding microtubule-dependent vesicular transport between specific membranes (117). PCylation of Rab1 may inhibit fusion of early secretory events involving vesicular transport on microtubules with ER-derived vesicles (117). AnkX also attaches a phosphocholine moiety to Rab35, a member of the Rab1 family which regulates the sorting of cargo from early endosomes. Modulation of Rab35 function results

in enlarged early endosomes (118). Thus, phosphocholination of Rab1 and Rab35 by AnkX is necessary for disrupting the activities of host membrane transport proteins and for efficient inhibition of the acquisition of the endosomal marker LAMP1 (117).

To counteract AnkX-mediated modification on Rab1, *Legionella* secretes Lem3, a dephosphorylcholinase, which reverses the post-translational modification on Rab1, making it more accessible to GEFs such as DrrA/SidM (119). Another *Legionella* effector that is important in the remodeling of the membrane is RalF. RalF is essential for recruiting the host GTPase ADP-ribosylation factor 1 (ARF1) to the phagosome and activating it via its ARF GEF activity (120). ARF1 is an enticing target for *Legionella* because it regulates transport of vesicles between the ER and Golgi apparatus (121).

Other *Legionella* effectors that have been shown to redirect the recruitment of ER-derived vesicles to the phagosome include SidJ, SidP, SidC, and subversion of eukaryotic traffic protein A (SetA) (122–124). SidJ redirects the recruitment of ER-derived vesicles to the *Legionella* phagosome (123). SidP is a PI phosphatase that hydrolyzes PI3P and PI(3,5)P<sub>2</sub>, promoting the evasion of the endocytic pathway by the phagosome (124). SidC anchors to the membrane via binding of PI4P, a marker of secretory-vesicle trafficking (106), and SidC is suggested to function as a bacterial tethering factor as *Legionella* lacking the *sidC* gene alter the recruitment of ER-derived vesicles to the phagosome (122). The exact mechanisms by which SidJ, SidP, and SidC recruit ER-derived vesicles, and the identity of their host targets, remain unknown. Lastly, SetA, a glycosyltransferase, modifies vesicular trafficking by attaching glucose moieties to conserved threonine residues within the catalytic region of host targets (125). SetA also anchors to the phagosomal membrane by binding to PIs (125). The effect of this modification remains unknown, but it is suggested to involve disruption of host targets and signaling events. The rerouting of

ER-derived vesicles to the surface of phagosomes harboring *Legionella* is suggested as a potential downstream effect of glycosylation catalyzed by SetA (126).

## CONCLUDING REMARKS

To perpetuate their reign, several infectious agents highjack circulating macrophages which paradoxically serve as both the first line of defense against microbial infections as well as the pathogens' natural habitat. In this review, we show that the ability of Gram-negative *Legionella*, *Salmonella*, *Shigella*, and *Yersinia*, as well as the acid-fast actinomycete *Mycobacterium*, to circumvent the phagocytes' bactericidal activity and perturb the host killing machinery is mediated by effectors injected into the cytoplasm of the host by specialized secretion systems. These secreted virulence factors confer remarkable resilience to pathogens by exerting functional redundancy with each other and facilitating and maximizing host cell invasion, replication, and intracellular survival.

The host-pathogen interactome is a recent field of study and requires further scrutiny. Much remains unknown regarding the cellular functions of the effectors implicated, their host targets, and their mechanisms of action. Understanding the approach and dynamics by which microbial proteins execute their functions will greatly increase our understanding of the mechanisms employed by pathogens to alter host cell physiology. The characterization of bacterial secreted proteins continues to be a major focus of future research. Newly acquired knowledge is crucial for the development of vaccines and therapeutic intervention against established and emerging infectious diseases.

## ACKNOWLEDGMENTS

Funding for this study was provided by the Canadian Institute of Health Research Operating Grant MOP-106622, the British Columbia

Lung Association, and the TB Veterans Association. We thank Stefan Szary for his help with illustrations and Joseph Chao and Jeffrey Helm for their useful comments.

## CITATION

Poirier V, Av-Gay Y. 2015. Intracellular growth of bacterial pathogens: the role of secreted effector proteins in the control of phagocytosed microorganisms. *Microbiol Spectrum* 3(6):VMBF-0003-2014.

## REFERENCES

1. **World Health Organization.** 2015. *The Top 10 Causes of Death*. Fact sheet No. 310. <http://www.who.int/mediacentre/factsheets/fs310/en/>.
2. **Guerrant RL, Blackwood BL.** 1999. Threats to global health and survival: the growing crises of tropical infectious diseases: our "unfinished agenda." *Clin Infect Dis* 28:966–986.
3. **Butler JC, Crengle S, Cheek JE, Leach AJ, Lennon D, O'Brien KL, Santosham M.** 2001. Emerging infectious diseases among indigenous peoples. *Emerg Infect Dis* 7(Suppl 3):554–555.
4. **Bliska JB, Copass MC, Falkow S.** 1993. The *Yersinia pseudotuberculosis* adhesin YadA mediates intimate bacterial attachment to and entry into HEp-2 cells. *Infect Immun* 61:3914–3921.
5. **Cascales E, Christie PJ.** 2003. The versatile bacterial type IV secretion systems. *Nat Rev Microbiol* 1:137–149.
6. **Backert S, Meyer TF.** 2006. Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr Opin Microbiol* 9:207–217.
7. **Saier MHJ.** 2006. Protein secretion systems in Gram-negative bacteria. *Microbe* 1:414–419.
8. **Yen M-R, Peabody CR, Partovi SM, Zhai Y, Tseng Y-H, Saier MHJ.** 2002. Protein-translocating outer membrane porins of Gram-negative bacteria. *Biochim Biophys Acta* 1562: 6–31.
9. **Thanassi DG, Bliska JB, Christie PJ.** 2012. Surface organelles assembled by secretion systems of Gram-negative bacteria: diversity in structure and function. *FEMS Microbiol Rev* 36:1046–1082.
10. **Abdallah AM, Gey van Pittius NC, Champion PA, Cox J, Luijckx BJ, Vandenbroucke-Grauls CM, Appelmelk BJ, Bitter W.** 2007. Type VII secretion: mycobacteria show the way. *Nat Rev Microbiol* 5:883–891.
11. **Lenz LL, Mohammadi S, Geissler A, Portnoy DA.** 2003. SecA2-dependent secretion of autolytic enzymes promotes *Listeria monocytogenes* pathogenesis. *Proc Natl Acad Sci USA* 100: 12432–12437.
12. **Stanier RY, Adelberg EA, Ingraham JL.** 1976. *The Microbial World*, 4th ed. Prentice-Hall, Englewood Cliffs, NJ.
13. **Coombes BK, Finlay BB.** 2005. Insertion of the bacterial type III translocon: not your average needle stick. *Trends Microbiol* 13:92–95.
14. **Bitter W, Houben EN, Bottai D, Brodin P, Brown EJ, Cox JS, Derbyshire K, Fortune SM, Gao LY, Liu J, Gey van Pittius NC, Pym AS, Rubin EJ, Sherman DR, Cole ST, Brosch R.** 2009. Systematic genetic nomenclature for type VII secretion systems. *PLoS Pathog* 5: e1000507. doi:10.1371/journal.ppat.1000507.
15. **Stanley SA, Raghavan S, Hwang WW, Cox JS.** 2003. Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system. *Proc Natl Acad Sci USA* 100:13001–13006.
16. **Guinn KM, Hickey MJ, Mathur SK, Zake KL, Grotzke JE, Lewinsohn DM, Smith S, Sherman DR.** 2004. Individual RD1-region genes are required for export of ESAT-6/CFP-10 and for virulence of *Mycobacterium tuberculosis*. *Mol Microbiol* 51:359–370.
17. **Abdallah AM, Verboom T, Hannes F, Safi M, Strong M, Eisenberg D, Musters RJ, Vandenbroucke-Grauls CM, Appelmelk BJ, Luijckx BJ, Bitter W.** 2006. A specific secretion system mediates PPE41 transport in pathogenic mycobacteria. *Mol Microbiol* 62:667–679.
18. **Flannagan RS, Jaumouillé V, Grinstein S.** 2012. The cell biology of phagocytosis. *Annu Rev Pathol* 7:61–98.
19. **Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG.** 1994. Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 263:678–681.
20. **Fraser DW, Tsai TR, Orenstein W, Parkin WE, Beecham HJ, Sharrar RG, Harris J, Mallison GF, Martin SM, McDade JE, Shepard CC, Brachman PS.** 1977. Legionnaires' disease: description of an epidemic of pneumonia. *N Engl J Med* 297:1189–1197.
21. **Baess I.** 1979. Deoxyribonucleic acid relatedness among species of slowly-growing mycobacteria. *Acta Pathol Microbiol Scand B* 87: 221–226.
22. **Stenmark H.** 2009. Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol* 10:513–525.



23. Christoforidis S, Miaczynska M, Ashman K, Wilm M, Zhao L, Yip SC, Waterfield MD, Backer JM, Zerial M. 1999. Phosphatidylinositol-3-OH kinases are Rab5 effectors. *Nat Cell Biol* 1:249–252.
24. Fratti RA, Backer JM, Gruenberg J, Corvera S, Deretic V. 2001. Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. *J Cell Biol* 154:631–644.
25. Lemmon MA. 2003. Phosphoinositide recognition domains. *Traffic* 4:201–213.
26. Ellson C, Davidson K, Anderson K, Stephens LR, Hawkins PT. 2006. PtdIns3P binding to the PX domain of p40phox is a physiological signal in NADPH oxidase activation. *EMBO J* 25:4468–4478.
27. Vieira OV, Harrison RE, Scott CC, Stenmark H, Alexander D, Liu J, Gruenberg J, Schreiber AD, Grinstein S. 2004. Acquisition of Hrs, an essential component of phagosomal maturation, is impaired by mycobacteria. *Mol Cell Biol* 24:4593–4604.
28. McBride HM, Rybin V, Murphy C, Giner A, Teasdale R, Zerial M. 1999. Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. *Cell* 98:377–386.
29. Jahn R, Scheller RH. 2006. SNAREs—engines for membrane fusion. *Nat Rev Mol Cell Biol* 7:631–643.
30. Chatterjee D, Khoo KH. 1998. Mycobacterial lipoarabinomannan: an extraordinary lipoheteroglycan with profound physiological effects. *Glycobiology* 8:113–120.
31. Malik ZA, Denning GM, Kusner DJ. 2000. Inhibition of Ca<sup>2+</sup> signalling by *Mycobacterium tuberculosis* is associated with reduced phagosome-lysosome fusion and increased survival within human macrophages. *J Exp Med* 191:287–302.
32. Vergne I, Chua J, Lee HH, Lucas M, Belisle J, Deretic V. 2005. Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 102:4033–4038.
33. Simonsen A, Gaullier JM, D'Arrigo A, Stenmark H. 1999. The Rab5 effector EEA1 interacts directly with syntaxin-6. *J Biol Chem* 274:28857–28860.
34. Ku B, Lee KH, Park WS, Yang CS, Ge J, Lee SG, Cha SS, Shao F, Heo WD, Jung JU, Oh BH. 2012. VipD of *Legionella pneumophila* targets activated Rab5 and Rab22 to interfere with endosomal trafficking in macrophages. *PLoS Pathog* 8:e1003082. doi:10.1371/journal.ppat.1003082.
35. Shohdy N, Efe JA, Emr SD, Shuman HA. 2005. Pathogen effector protein screening in yeast identifies *Legionella* factors that interfere with membrane trafficking. *Proc Natl Acad Sci USA* 102:4866–4871.
36. 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.
37. Madan R, Krishnamurthy G, Mukhopadhyay A. 2008. SopE-mediated recruitment of host Rab5 on phagosomes inhibits *Salmonella* transport to lysosomes. *Methods Mol Biol* 445:417–437.
38. Mukherjee K, Parashuraman S, Raje M, Mukhopadhyay A. 2001. SopE acts as a 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.
39. 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.
40. Alpuche-Aranda CM, Racoosin EL, Swanson JA, Miller SI. 1994. *Salmonella* stimulate macrophage macropinocytosis and persist within spacious phagosomes. *J Exp Med* 179:601–608.
41. Alvarez-Dominguez C, Barbieri AM, Berón W, Wandinger-Ness A, Stahl PD. 1996. Phagocytosed live *Listeria monocytogenes* influences Rab5-regulated *in vitro* phagosome-endosome fusion. *J Biol Chem* 271:13834–13843.
42. Via LE, Deretic D, Ulmer RJ, Hibler NS, Huber LA, Deretic V. 1997. Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by Rab5 and Rab7. *J Biol Chem* 272:13326–13331.
43. Darsow T, Reider SE, Emr SD. 1997. A multi-specificity syntaxin homologue, Vam3p, essential for autophagic and biosynthetic protein transport to the vacuole. *J Cell Biol* 138:517–529.
44. Price A, Wickner W, Ungermann C. 2000. Proteins needed for vesicle budding from the golgi complex are also required for the docking step of homotypic vacuole fusion. *J Cell Biol* 148:1223–1229.
45. 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.
46. Shotland Y, Krämer H, Groisman EA. 2003. The *Salmonella* SpiC protein targets

- the mammalian Hook3 protein function to alter cellular trafficking. *Mol Microbiol* **49**:1565–1576.
47. Di Paolo G, De Camilli P. 2006. Phosphoinositides in cell regulation and membrane dynamics. *Nature* **443**:651–657.
  48. Marcus SL, Knodler LA, Finlay BB. 2002. *Salmonella enterica* serovar Typhimurium effector SigD/SopB is membrane-associated and ubiquitinated inside host cells. *Cell Microbiol* **4**:435–446.
  49. Bach H, Papavinasandaram KG, Wong D, Hmama Z, Av-Gay Y. 2008. *Mycobacterium tuberculosis* virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B. *Cell Host Microbe* **3**:316–322.
  50. Banta LM, Robinson JS, Klionsky DJ, Emr SD. 1988. Organelle assembly in yeast: characterization of yeast mutants defective in vacuolar biogenesis and protein sorting. *J Cell Biol* **107**:1369–1383.
  51. Mehra A, Zahra A, Thompson V, Sirisaengtaksin N, Wells A, Porto M, Köster S, Penberthy K, Kubota Y, Dricot A, Rogan D, Vidal M, Hill DE, Bean AJ, Philips JA. 2013. *Mycobacterium tuberculosis* type VII secreted effector EsxH targets host ESCRT to impair trafficking. *PLoS Pathog* **9**:e1003734. doi:10.1371/journal.ppat.1003734.
  52. Katzmann DJ, Odorizzi G, Emr SD. 2002. Receptor downregulation and multivesicular-body sorting *Nat Rev Mol Cell Biol* **3**:893–905.
  53. Xu J, Laine O, Masciocchi M, Manoranjan J, Smith J, Du SJ, Edwards N, Zhu X, Fenselau C, Gao LY. 2007. A unique mycobacterium ESX-1 protein co-secreted with CFP-10/ESAT-6 and is necessary for inhibiting phagosome maturation. *Mol Microbiol* **66**:3787–3800.
  54. Hunter RL, Olsen MR, Jagannath C, Actor JK. 2006. Multiple roles of cord factor in the pathogenesis of primary, secondary, and cavity tuberculosis, including a revised description of the pathology of secondary disease. *Ann Clin Lab Sci* **36**:371–386.
  55. Hoekstra D, Düzgünes N, Wilschut J. 1985. Agglutination and fusion of globoside GL-4 containing phospholipid vesicles mediated by lectins and calcium ions. *Biochemistry* **24**:565–572.
  56. Spargo BJ, Crowe LM, Ionedo T, Beaman BL, Crowe JH. 1991. Cord factor (alpha,alpha-trehalose 6,6'-dimycolate) inhibits fusion between phospholipid vesicles. *Proc Natl Acad Sci USA* **88**:737–740.
  57. Rosqvist R, Bölin I, Wolf-Watz H. 1988. Inhibition of phagocytosis in *Yersinia pseudotuberculosis*: a virulence plasmid-encoded ability involving the Yop2b protein. *Infect Immun* **56**:2139–2143.
  58. Pujol C, Bliska JB. 2003. The ability to replicate in macrophages is conserved between *Yersinia pestis* and *Yersinia pseudotuberculosis*. *Infect Immun* **71**:5892–5829.
  59. Tsukano H, Kura F, Inoue S, Sato S, Izumiya H, Yasuda T, Watanabe H. 1999. *Yersinia pseudotuberculosis* blocks the phagosomal acidification of B10.A mouse macrophages through the inhibition of vacuolar H(+)-ATPase activity. *Microb Pathog* **27**:253–263.
  60. Tabrizi SN, Robins-Browne RM. 1992. Influence of a 70 kilobase virulence plasmid on the ability of *Yersinia enterocolitica* to survive phagocytosis *in vitro*. *Microb Pathog* **13**:171–179.
  61. Finlay BB, Falkow S. 1997. Common themes in microbial pathogenicity revisited. *Microbiol Mol Biol Rev* **61**:136169.
  62. Duclos S, Desjardins M. 2000. Subversion of a young phagosome: the survival strategies of intracellular pathogens. *Cell Microbiol* **2**:365–377.
  63. Straley SC, Harmon PA. 1984. *Yersinia pestis* grows within phagolysosomes in mouse peritoneal macrophages. *Infect Immun* **45**:655–659.
  64. Holden DW. 2002. Trafficking of the *Salmonella* vacuole in macrophages. *Traffic* **3**:161–169.
  65. Hackam DJ, Rotstein OD, Zhang WJ, Demarex N, Woodside M, Tsai O, Grinstein S. 1997. Regulation of phagosomal acidification. Differential targeting of Na<sup>+</sup>/H<sup>+</sup> exchangers, Na<sup>+</sup>/K<sup>+</sup>-ATPases, and vacuolar-type H<sup>+</sup>-ATPases. *J Biol Chem* **272**:29810–29820.
  66. Wong D, Bach H, Hmama Z, Av-Gay Y. 2011. *Mycobacterium tuberculosis* protein tyrosine phosphatase A disrupts phagosome acidification by exclusion of host vacuolar H<sup>+</sup>-ATPase. *Proc Natl Acad Sci USA* **108**:19371–196.
  67. Xu L, Shen X, Bryan A, Banga S, Swanson MS, Luo ZQ. 2010. Inhibition of host vacuolar H<sup>+</sup>-ATPase activity by a *Legionella pneumophila* effector. *PLoS Pathog* **6**:e1000822. doi:10.1371/journal.ppat.1000822.
  68. Prost LR, Daley ME, Le Sage V, Bader MW, Le Moual H, Klevit RE, Miller SI. 2007. Activation of the bacterial sensor kinase PhoQ by acidic pH. *Mol Cell* **26**:165–174.
  69. Nikolaus T, Deiwick J, Rapp C, Freeman JA, Schröder W, Miller SI, Hensel M. 2001. SseBCD proteins are secreted by the type III secretion system of *Salmonella* pathogenicity island 2 and function as a translocon. *J Bacteriol* **183**:6036–6045.
  70. Scott CC, Botelho RJ, Grinstein S. 2003. Phagosome maturation: a few bugs in the system. *J Membr Biol* **193**:137–152.

71. 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.
72. 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.
73. Hayward RD, Koronakis V. 1999. Direct nucleation and bundling of actin by the SipC protein of invasive *Salmonella*. *EMBO J* 18:4926–4934.
74. 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.
75. 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.
76. Braun V, Wong A, Landekic M, Hong WJ, Grinstein S, Brumell JH. 2010. Sorting nexin 3 (SNX3) is a component of a tubular endosomal network induced by *Salmonella* and involved in maturation of the *Salmonella*-containing vacuole. *Cell Microbiol* 12:1352–1367.
77. Drecktrah D, Levine-Wilkinson S, Dam T, Winfree S, Knodler LA, Schroer TA, Steele-Mortimer O. 2008. Dynamic behavior of *Salmonella*-induced membrane tubules in epithelial cells. *Traffic* 9:2117–2129.
78. Rajashekar R, Liebl D, Seitz A, Hensel M. 2008. Dynamic remodeling of the endosomal system during formation of *Salmonella*-induced filaments by intracellular *Salmonella enterica*. *Traffic* 9:2100–2116.
79. Husebye H, Aune MH, Stenvik J, Samstad E, Skjeldal F, Halaas O, Nilsen NJ, Stenmark H, Latz E, Lien E, Mollnes TE, Bakke O, Espevik T. 2010. The Rab11a GTPase controls Toll-like receptor 4-induced activation of interferon regulatory factor-3 on phagosomes. *Immunity* 33:583–596.
80. 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.
81. 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 USA* 103:13497–13502.
82. Kuhle V, Hensel M. 2002. SseF and SseG are translocated effectors of the type III secretion system of *Salmonella* pathogenicity island 2 that modulate aggregation of endosomal compartments. *Cell Microbiol* 4:813–824.
83. Vale RD, Reese TS, Sheetz MP. 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* 42:39–50.
84. 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.
85. 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.
86. Cai D, McEwen DP, Martens JR, Meyhofer E, Verhey KJ. 2009. Single molecule imaging reveals differences in microtubule track selection between kinesin motors. *PLoS Biol* 7:e1000216. doi:10.1371/journal.pbio.1000216.
87. 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.
88. Kuhle V, Abrahams GL, Hensel M. 2006. Intracellular *Salmonella enterica* redirect exocytic transport processes in a *Salmonella* pathogenicity island 2-dependent manner. *Traffic* 7:716–730.
89. Franco IS, Shohdy N, Shuman HA. The *Legionella pneumophila* effector VipA is an actin nucleator that alters host cell organelle trafficking. *PLoS Pathog* 8:e1002546. doi:10.1371/journal.ppat.1002546.
90. Stamm LM, Morisaki JH, Gao LY, Jeng RL, McDonald KL, Roth R, Takeshita S, Heuser J, Welch MD, Brown EJ. 2003. *Mycobacterium marinum* escapes from phagosomes and is propelled by actin-based motility. *J Exp Med* 198:1361–1368.
91. Goldberg MB. 2001. Actin-based motility of intracellular microbial pathogens. *Microbiol Mol Biol Rev* 65:595–626.
92. Moors MA, Levitt B, Youngman P, Portnoy DA. 1999. Expression of listeriolysin O and ActA by intracellular and extracellular *Listeria monocytogenes*. *Infect Immun* 67:131–139.
93. Goldberg MB, Theriot JA, Sansonetti PJ. 1994. Regulation of surface presentation of

- IcsA, a *Shigella* protein essential to intracellular movement and spread, is growth phase dependent. *Infect Immun* **62**:5664–5668.
94. Niebuhr K, Giuriato S, Pedron T, Philpott DJ, Gaits F, Sable J, Sheetz MP, Parsot C, Sansonetti PJ, Payraastre 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.
  95. Ramel D, Lagarrigue F, Pons V, Mounier J, Dupuis-Coronas S, Chicanne G, Sansonetti PJ, Gaits-Iacovoni F, Tronchère H, Payraastre B. 2011. *Shigella flexneri* infection generates the lipid PI5P to alter endocytosis and prevent termination of EGFR signaling. *Sci Signal* **4**:ra61.
  96. 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.
  97. Blocker A, Gounon P, Larquet E, Niebuhr K, Cabiaux V, Parsot C, Sansonetti P. 1999. The tripartite type III secretin of *Shigella flexneri* inserts IpaB and IpaC into host membranes. *J Cell Biol* **147**:683–693.
  98. 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.
  99. Mounier J, Laurent V, Hall A, Fort P, Carlier MF, Sansonetti PJ, Egile C. 1999. Rho family GTPases control entry of *Shigella flexneri* into epithelial cells but not intracellular motility. *J Cell Sci* **112**:2069–2080.
  100. Fernandez-Prada CM, Hoover DL, Tall BD, Hartman AB, Kopelowitz J, Venkatesan MM. 2000. *Shigella flexneri* IpaH(7.8) facilitates escape of virulent bacteria from the endocytic vacuoles of mouse and human macrophages. *Infect Immun* **68**:3608–3619.
  101. Portnoy DA, Jacks PS, Hinrichs DJ. 1988. Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. *J Exp Med* **167**:1459–1471.
  102. Smith GA, Marquis H, Jones S, Johnston NC, Portnoy DA, Goldfine H. 1995. The two distinct phospholipases C of *Listeria monocytogenes* have overlapping roles in escape from a vacuole and cell-to-cell spread. *Infect Immun* **63**:4231–4237.
  103. Tilney LG, Harb OS, Connelly PS, Robinson CG, Roy CR. 2001. How the parasitic bacterium *Legionella pneumophila* modifies its phagosome and transforms it into rough ER: implications for conversion of plasma membrane to the ER membrane. *J Cell Sci* **114**:4637–4650.
  104. Kagan JC, Roy CR. 2002. *Legionella* phagosomes intercept vesicular traffic from endoplasmic reticulum exit sites. *Nat Cell Biol* **4**:945–954.
  105. Hsu F, Zhu W, Brennan L, Tao L, Luo ZQ, Mao Y. 2012. Structural basis for substrate recognition by a unique *Legionella* phosphoinositide phosphatase. *Proc Natl Acad Sci USA* **109**:13567–13572.
  106. Weber SS, Ragaz C, Reus K, Nyfeler Y, Hilbi H. 2006. *Legionella pneumophila* exploits PI(4)P to anchor secreted effector proteins to the replicative vacuole. *PLoS Pathog* **2**:e46.
  107. Conover GM, Derré I, Vogel JP, Isberg RR. 2003. The *Legionella pneumophila* LidA protein: a translocated substrate of the Dot/Icm system associated with maintenance of bacterial integrity. *Mol Microbiol* **48**:305–321.
  108. Machner MP, Isberg RR. 2006. Targeting of host Rab GTPase function by the intravacuolar pathogen *Legionella pneumophila*. *Dev Cell* **11**:47–56.
  109. Moyer BD, Allan BB, Balch WE. 2001. Rab1 interaction with a GM130 effector complex regulates COPII vesicle cis-Golgi tethering. *Traffic* **2**:268–276.
  110. Kagan JC, Stein MP, Pypaert M, Roy CR. 2004. *Legionella* subvert the functions of Rab1 and Sec22b to create a replicative organelle. *J Exp Med* **199**:1201–1211.
  111. Murata T, Delprato A, Ingmundson A, Toomre DK, Lambright DG, Roy CR. 2006. The *Legionella pneumophila* effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nat Cell Biol* **8**:971–977.
  112. Müller MP, Peters H, Blümer J, Blankenfeldt W, Goody RS, Itzen A. 2010. The *Legionella* effector protein DrrA AMPylates the membrane traffic regulator Rab1b. *Science* **329**:946–949.
  113. Chen J, de Felipe KS, Clarke M, Lu H, Anderson OR, Segal G, Shuman HA. 2004. *Legionella* effectors that promote nonlytic release from protozoa. *Science* **303**:1358–1361.
  114. Ingmundson A, Delprato A, Lambright DG, Roy CR. 2007. *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. *Nature* **450**:365–369.
  115. Tan Y, Luo ZQ. 2011. *Legionella pneumophila* SidD is a deAMPylase that modifies Rab1. *Nature* **475**:506–509.
  116. Mukherjee S, Liu X, Arasaki K, McDonough J, Galán JE, Roy CR. 2011. Modulation of Rab GTPase function by a protein phosphocholine transferase. *Nature* **477**:103–106.
  117. Pan X, Lührmann A, Satoh A, Laskowski-Arce MA, Roy CR. 2008. Ankyrin repeat proteins comprise a diverse family of bacterial type IV effectors. *Science* **320**:1651–1654.

118. Allaire PD, Marat AL, Dall'Armi C, Di Paolo G, McPherson PS, Ritter B. 2010. The Connecdenn DENN domain: a GEF for Rab35 mediating cargo-specific exit from early endosomes. *Mol Cell* **37**:370–382.
119. Tan Y, Arnold RJ, Luo ZQ. 2011. *Legionella pneumophila* regulates the small GTPase Rab1 activity by reversible phosphorylcholine. *Proc Natl Acad Sci USA* **108**:21212–21217.
120. Nagai H, Kagan JC, Zhu X, Kahn RA, Roy CR. 2002. A bacterial guanine nucleotide exchange factor activates ARF on *Legionella* phagosomes. *Science* **295**:679–682.
121. Robinson CG, Roy CR. 2006. Attachment and fusion of endoplasmic reticulum with vacuoles containing *Legionella pneumophila*. *Cell Microbiol* **8**:793–805.
122. Ragaz C, Pietsch H, Urwyler S, Tiaden A, Weber SS, Hilbi H. 2008. 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.
123. Liu Y, Luo ZQ. 2007. The *Legionella pneumophila* effector SidJ is required for efficient recruitment of endoplasmic reticulum proteins to the bacterial phagosome. *Infect Immun* **75**:592–603.
124. Toulabi L, Wu X, Cheng Y, Mao Y. 2013. Identification and structural characterization of a *Legionella* phosphoinositide phosphatase. *J Biol Chem* **288**:24518–24527.
125. Jank T, Böhmer KE, Tzivelekidis T, Schwan C, Belyi Y, Aktories K. 2012. Domain organization of *Legionella* effector SetA. *Cell Microbiol* **14**:852–868.
126. Heidtman M, Chen EJ, Moy MY, Isberg RR. 2009. Large-scale identification of *Legionella pneumophila* Dot/Icm substrates that modulate host cell vesicle trafficking pathways. *Cell Microbiol* **11**:230–248.