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How Bacteria Subvert Animal Cell Structure and Function

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Abstract

Bacterial pathogens encode a wide variety of effectors and toxins that hijack host cell structure and function. Of particular importance are virulence factors that target actin cytoskeleton dynamics critical for cell shape, stability, motility, phagocytosis, and division. In addition, many bacteria target organelles of the general secretory pathway (e.g., the endoplasmic reticulum and the Golgi complex) and recycling pathways (e.g., the endolysosomal system) to establish and maintain an intracellular replicative niche. Recent research on the biochemistry and structural biology of bacterial effector proteins and toxins has begun to shed light on the molecular underpinnings of these host-pathogen interactions. This exciting work is revealing how pathogens gain control of the complex and dynamic host cellular environments, which impacts our understanding of microbial infectious disease, immunology, and human cell biology.

Keywords

bacterial effectors; bacterial toxins; actin cytoskeleton; Golgi complex; endosomal/lysosomal trafficking

OVERVIEW

Bacteria interact and take control of their environment by delivering macromolecules across the inner membranes and outer membranes of gram-positive and gram-negative bacteria, respectively. At least seven secretory systems (SS) have evolved for this purpose. However, the type 3 SS (T3SS), type 4 SS (T4SS), and type 6 SS (T6SS) have the unique ability to translocate virulent effector proteins from the bacterial cytoplasm directly to the cytoplasm of host cells (Costa et al. 2015, Hayes et al. 2010). These three secretion systems are found primarily in pathogenic bacteria, and the loss of their protein delivery capacities results in avirulent phenotypes. By extension, the function of secreted effector proteins is essential to both animal and plant infectious disease.

The term effector protein is a generic description of any bacterial protein delivered into the host cytoplasm by the T3SS, T4SS, and T6SS apparatus. This term also distinguishes the mode of host cell delivery from that of classic toxins that are first secreted into the

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extracellular space prior to host cell uptake by endocytic-type mechanisms. However, there are several critical differences between effectors and toxins. Unlike large, multidomain bacterial toxins, effectors are typically small proteins composed of a short secretory signal, which is required for host translocation and is linked to a minimal enzymatic or functional domain (Dean 2011). Once delivered into the host cell, effector proteins recognize specific substrates and typically catalyze chemical reactions that disrupt substrate functions. Although the inhibitory activities of bacterial effector proteins have deleterious effects on host cell architecture and signal transduction, many effector proteins have evolved to directly activate substrates for the purpose of reprogramming cell behavior. These reprogramming events allow pathogens to access new cell sites, such as the cytoplasm or intracellular vacuole, that can promote dissemination across tissue barriers and/or evasion of the host immune defense systems. Thus, the action of bacterial effector proteins allows pathogens to exploit host environments that are typically inaccessible to normal flora.

In this review, our goal is to distill the wealth of information on bacterial effector proteins and toxins, with a specific emphasis on detailing the molecular mechanisms of substrate recognition and the chemical modifications that alter host cell structure and function. Although some historical context is discussed, we primarily emphasize new studies that reveal how pathogens gain control of two major structural elements of the host cell: the actin cytoskeleton and intracellular organelles.

THE ACTIN CYTOSKELETON: A TARGET OF BACTERIAL PATHOGENS THAT CONTROLS HOST STRUCTURE AND FUNCTION

Actin is one of the most abundant proteins in eukaryotic cells and is involved in multiple essential cell processes, including motility, division, organelle function, and the maintenance of cell architecture. As such, the regulatory components of actin filaments are attractive targets of bacterial pathogens. Microbial perturbation of actin dynamics often results in profound changes in host cell structure that can result in opposing phenotypic outcomes, such as the induction versus inhibition of phagocytosis. Actin is a monomeric ATPase (G-actin) that can undergo cycles of spontaneous self-assembly into filaments (F-actin), ATP hydrolysis, and depolymerization. In the cell, the transition from G-actin to F-actin is tightly controlled by numerous actin-binding proteins (ABPs) and their upstream activators, which together produce all actin architectures required for cell function. Actin dynamics are controlled by the activity of the Rho family of small GTPases (Spiering & Hodgson 2011). Once activated by upstream stimuli, such as growth factor receptor engagement, Rho-family GTPases signal to downstream effectors, including nucleation-promoting factors (NPFs). NPFs in turn activate the Arp2/3 complex, the actin nucleator responsible for the generation of branched actin filaments. Interestingly, pathogens have evolved toxins and effector proteins to directly mimic the function of many ABPs and their regulators. These processes can be broadly categorized into three classes of pathogenic regulators: (a) toxins/ effectors that directly regulate actin filament assembly, bundling, or depolymerization; (b) toxins/ effector proteins that indirectly regulate F-actin by mimicking actin nucleators or their upstream NPFs; and (c) toxins/ effectors that target the Rho-family GTPases, master regulators of signal transduction to the actin cytoskeleton (Figure 1).

Bacterial Effectors Directly Promote Actin Polymerization by Mimicking Host Factors

To precisely control the actin dynamics required for sophisticated cell processes, the nucleation of actin is tightly regulated within the cell. Actin polymerization into F-actin filaments begins with the formation of an actin nucleus, a stable complex of actin monomers that structurally resemble the arrangement of subunits in a filament. Once the actin nucleus forms, additional actin monomers readily bind to the barbed end of the filament, and elongation occurs. Because nucleus formation is the rate-limiting step in the polymerization reaction, pathogens have evolved effector proteins that promote actin nucleation. Perhaps the greatest insights into this process have occurred through the study of bacterial pathogens that hijack actin polymerization for intracellular motility and cell-to-cell spread. One of the best-studied pathogens in this regard, *Listeria monocytogenes*, activates host NPF Arp2/3 (discussed in more detail below). In contrast, several pathogens, including *Rickettsia* and some *Vibrio* species, secrete effector proteins that directly polymerize actin.

Rickettsia are gram-negative, obligate intracellular pathogens that infect humans via an arthropod vector; these pathogens are responsible for multiple diseases, including typhus and spotted fever. Like *Listeria*, *Rickettsia* depends upon actin-based motility for its intracellular propulsion. *Rickettsia* was the first pathogen found to bypass Arp2/3 activation for motility and instead encodes the autotransporter Sca2, a formin-like actin nucleator (Haglund et al. 2010, Madasu et al. 2013). Sca2 mimics formins in its ability to function as an elongation factor by remaining processively associated with the barbed end of the growing actin filament, by requiring profilin for efficient elongation, and by inhibiting actin filament-capping proteins. Recently, the N-terminal domain of Sca2 was crystallized and shown to adopt a previously uncharacterized fold that has a series of helix-loop-helix repeats and exhibits a crescent moon structure (Figure 2a) (Madasu et al. 2013). It is predicted that the C-terminal domain also contains helix-loop-helix repeats and adopts a crescent shape similar to that of host formins (Otomo et al. 2005). The interaction of the N- and C-terminal domains may result in a ringlike structure reminiscent of the ringlike structure of the formin FH2 homodimer. By extension, these domains are predicted to be responsible for organizing actin monomers into an actin nucleus. Sca2 also contains three WASP homology 2 (WH2) domains for actin monomer recruitment and proline-rich motifs (PRMs) for profilin-dependent elongation. Interestingly, *Rickettsia* motility requires Sca2 as well as RickA, a *Rickettsia* NPF, but these molecules act independently at different stages throughout the infection process (Reed et al. 2014).

The T3SS effectors VopL from *Vibrio parahaemolyticus* and VopF (a homolog of VopL) from *Vibrio cholerae* nucleate actin in vitro and, during infection, generate actin stress fibers and filopodia, respectively (Liverman et al. 2007, Yu et al. 2011). Recent structural insights into VopL have provided the strongest evidence for the mechanism by which actin nucleation occurs. VopL is a multidomain protein encoding a VopL C-terminal domain (VCD) and an array of three WH2 domains interspaced with PRMs. Although the VCD domain is sufficient for nucleation, its activity is greatly enhanced by the WH2 domains (Namgoong et al. 2011, Yu et al. 2011). The structure of the VCD dimer was solved in complex with three actin monomers, and this structure revealed that actin monomers bound by the VCD dimer are arranged in a manner similar to that of actin subunits found within a

short-pitch actin filament (Zahm et al. 2013). However, as Zahm et al. (2013) point out, the actin monomers are arranged in a confirmation that is not conducive for polymerization, suggesting that additional structural rearrangements required for filament elongation may be responsible for the rapid release of VopL from the growing filament. Competing models of VopL/F-actin structures have been proposed (Avvaru et al. 2015, Namgoong et al. 2011). Nevertheless, VopL-mediated actin nucleation occurs through the cooperation between its VCD actin organization domain and actin-binding WH2 domains. These distinct actin-binding and actin organization properties are shared among eukaryotic nucleation factors, including Arp2/3 and formins, indicating that VopL functionally mimics eukaryotic proteins.

Bundling and Cross-Linking Actin by Bacterial Toxins and Effectors

Actin filaments can be bundled into thick fibers or cross-linked into a weblike architecture that supports cell structures. Pathogens have evolved effectors and toxins to produce similar actin phenotypes for virulence. For example, the *V. parahaemolyticus* effector VopV is secreted by the T3SS2, and its ability to bind and bundle F-actin filaments is required for its enterotoxicity (Hiyoshi et al. 2011). VopV consists of a central long repetitive (LR) domain that confers these interaction capabilities, and recently, the structure of one of the repeat units found within the LR domain of VopV (VopVrep1) was solved in complex with F-actin (Nishimura et al. 2015). The 9.6-Å cryoEM structure revealed that VopVrep1 undergoes a disorder-to-order transition upon binding across the interstrand region of F-actin. Interestingly, the interstrand region is also the binding site for the *Salmonella* effector SipA, which is discussed in more detail below (Galkin et al. 2002).

Salmonella encodes two T3SS: the SPI-1 system for bacterial invasion and the SPI-2 system for intracellular survival and replication. The SPI-1 effectors SipA and SipC act cooperatively to mediate actin polymerization and actin filament bundling (McGhie et al. 2001) and, along with additional effectors such as SopE and SptP (discussed below), are responsible for *Salmonella* invasion. SipA promotes actin polymerization in several ways. First, SipA stabilizes F-actin and lowers the critical concentration for actin polymerization tenfold (Zhou et al. 1999). Electron microscopy revealed that the actin-binding fragment of SipA stabilizes filaments by binding along the interstrand region (Galkin et al. 2002). Second, SipA inhibits ADF/cofilin depolymerization through competitive binding. Finally, SipA inhibits and reverses gelsolin-mediated actin severing (McGhie et al. 2004). This multitude of activities are also coordinated with the action of SipC, a component of the *Salmonella* T3SS machinery (Kaniga et al. 1995a) that nucleates actin polymerization (Hayward & Koronakis 1999) and bundles actin filaments (Myeni & Zhou 2010). Although SipC oligomerization has been shown to be important for its nucleation properties (Chang et al. 2007), the lack of structural studies currently precludes the determination of its mechanism of nucleation. However, homologs of SipA and SipC are found in the intracellular pathogens *Shigella flexneri* and *Burkholderia pseudomallei* (Druar et al. 2008; Kaniga et al. 1995a,b), suggesting that these effectors may also be key factors in host cell invasion.

Bacterial Toxins and Effectors Catalyzing Actin Depolymerization

Historically, many of the most important bacterial toxins were found to directly depolymerize actin filaments by ADP-ribosylation. The C2 toxin from *Clostridium botulinum* (Aktories et al. 1986), the iota toxin from *Clostridium perfringens* (Stiles & Wilkins 1986), the *Clostridium difficile* transferase toxin (Popoff et al. 1988), the *Clostridium spiroforme* toxin (Simpson et al. 1989), the vegetative insecticidal proteins from *Bacillus cereus* (Han et al. 1999), and the *Salmonella* SPI-2 T3SS effector SpvB (Otto et al. 2000) have been shown to ADP-ribosylate G-actin. These toxins reversibly modify G-actin at Arg177 through the addition of an ADP-ribose moiety from NAD (Aktories & Wegner 1989). The functional result of this modification is the conversion of G-actin into a polymerization-deficient subunit. Incorporation of this modified G-actin into the growing filament effectively caps the growing filament, leading to subsequent filament depolymerization.

Bacterial Effectors Mimicking Actin Nucleation-Promoting Factors

It has long been known that intracellular pathogens such as *L. monocytogenes* propel themselves throughout eukaryotic cells on so-called actin comet tails (and can penetrate the plasma membrane and thereby move from cell to cell). This observation has been the subject of intense investigation over the past two decades and has revolutionized our understanding of actin filament dynamics (Pollard et al. 2000). Importantly, mechanistic studies on *Listeria* motility have been essential for defining the minimal components required for sustained actin polymerization. Such studies led to the first identification and activation mechanism of the Arp2/3 nucleation complex (Machesky et al. 1994, Welch et al. 1997) as well as the conceptual and experimental framework for the complete reconstitution of cell motility in vitro (Loisel et al. 1999). In addition to these pioneering studies, intracellular actin motility has been described for unrelated bacterial pathogens that activate actin nucleators (e.g., Arp2/3) or their upstream NPFs (e.g., N-WASP), including *S. flexneri* (Goldberg & Theriot 1995), *Burkholderia* (Benanti et al. 2015), and *Mycobacterium marinum* (Stamm et al. 2003). Extracellular pathogens also secrete T3SS effector proteins; notably, enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 effectors EspF and EspFu (also known as TccP) activate N-WASP (Alto et al. 2007, Sallee et al. 2008).

Bacteria Targeting Rho-Family GTPases

Rho-family GTPases are small-molecular guanine nucleotide-binding proteins that function as molecular switches, existing in an inactive GDP-bound state and an active GTP-bound state. Controlling the interconversion between these two states are guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs activate Rho-family GTPases by mediating the exchange of GDP for GTP and GAPs, which inactivate GTPases by promoting the hydrolysis of the bound GTP to GDP (recently reviewed in Cherfils & Zeghouf 2013). In their GTP-bound state, Rho-family GTPases activate their downstream effectors, including the WASP family of NPFs (Rohatgi et al. 1999). Thus, the cycles of guanine nucleotide exchange and hydrolysis are directly coupled to the induction of actin polymerization. In addition, the location of Rho GTPase function is controlled by long-chain fatty acid modifications of the C-terminal CaaX motif; such modifications target the

GTPases to the plasma membrane and various intracellular organelles. Remarkably, bacterial pathogens have evolved toxins and effector proteins to target nearly every step of the Rho GTPase signaling cascade through molecular mimicry of GTPase regulatory proteins [GEFs, GAPs, and guanine nucleotide dissociation inhibitors (GDIs)] and through posttranslational modifications (PTMs) (discussed in detail below).

Bacterial GEFs

The first bacterial GEF identified was SopE, a T3SS effector from *Salmonella* species that mediates bacterial invasion (Hardt et al. 1998). SopE activates Rac1 and, along with its later identified homolog SopE2, can also activate Cdc42 to induce actin polymerization and ultimately internalization of the invading bacterium (Friebe et al. 2001, Rudolph et al. 1999, Stender et al. 2000). Homologs of SopE include CopE, which is found in *Chromobacterium violaceum* (Miki et al. 2011), and BopE, which is encoded by *B. pseudomallei* (Stevens et al. 2003). Structural studies on SopE showed that, unlike the eukaryotic Rho-family GTPase GEFs that contain a Dbl-homology (DH) domain or Dock Homology region 2 (DHR-2) domains (Yang et al. 2009, Yu et al. 2010), SopE adopts a V-shaped fold that consists of two intersecting bundles of α -helices connected by an extended catalytic loop (Figure 2b) (Buchwald et al. 2002). Although structurally distinct, both eukaryotic and bacterial GEFs induce similar nucleotide-free transition states that are required for efficient nucleotide exchange. More than a decade later, a second family of bacterial GEFs was identified. This family shares an invariant Trp-X-X-X-Glu (WxxxE) signature motif, and members include IpgB1 and IpgB2 from *Shigella*, SifA and SifB from *Salmonella*, and Map and EspM from *E. coli*. Although the SopE-type GEFs and WxxxE GEFs share no sequence homology, they adopt nearly identical V-shaped structures (Figure 2b). Clearly, these families have diverged from a common evolutionary ancestor, yet surprisingly they are structurally distinct from eukaryotic GEFs (Figure 2b). Recent studies suggest that bacterial GEFs are further endowed with accessory motifs that drive their subcellular localization and their ability to locally amplify actin polymerization (Orchard et al. 2012). This is an open area of investigation that could help explain how effector proteins secreted at very low concentration can induce robust changes in cell architecture and signal transduction.

Bacterial GAPs

Pathogenic bacteria have also evolved effector proteins that mimic GAPs to dampen Rho-family GTPase signaling. One of the most intriguing functions of bacterial GAPs is their ability to suppress bacterial GEFs required for pathogenic invasion. As *Salmonella* is an intracellular organism, profound and long-lasting Rho-family GTPase activation by SopE and SopE2 would be counterproductive to *Salmonella*'s pathogenicity. Therefore, *Salmonella* has evolved the GAP SptP to reverse the extensive effector-mediated actin polymerization following internalization (Fu & Galan 1999, Stebbins & Galan 2000). The translocation of SopE prior to SptP coupled with the relatively rapid degradation of SopE after translocation confers a sophisticated degree of temporal regulation of Rho-family GTPase signaling during infection (Kubori & Galan 2003, Van Engelenburg & Palmer 2008, Winnen et al. 2008). Much like eukaryotic GAPs, bacterial GAPs commonly use a conserved arginine finger to induce efficient GTP hydrolysis, switching the Rho-family GTPases into their

inactive state. Bacterial GAPs are found encoded in *Salmonella* spp., *Yersinia* spp., and *Pseudomonas aeruginosa* (Aili et al. 2002, Andor et al. 2001, Sundin et al. 2001).

Regulation of GTPase Localization by Bacterial GDIs and Prenyl-Hydrolases

In addition to directly controlling the nucleotide cycling of the Rho-family GTPases, bacterial pathogens have evolved the ability to remove Rho-family GTPases from cell membranes. Rho-family GTPases are acylated at their C terminus, and proper membrane localization is critical for GTPase function (Cherfils & Zeghouf 2013). When the acyl group is embedded into the membrane, Rho-family GTPases are able to interact with their GEFs and, once activated, can engage downstream signaling molecules. GDIs carry out the removal of a GTPase from the membrane through the sequestration of the prenylated CaaX motif (Hoffman et al. 2000, Scheffzek et al. 2000). The *Yersinia* effector YopO (also known as YpkA) acts as a GDI mimic for Rac1 and RhoA (Juris et al. 2000, Prehna et al. 2006). Furthermore, another *Yersinia* effector, YopT, functions as a cysteine protease and disrupts the actin cytoskeleton by cleaving lipid-modified CaaX sequence from the GTPase (Shao et al. 2002). Liberation of Rho GTPases from the membrane irreversibly inhibits signaling to the actin cytoskeleton.

Posttranslational Modification of GTPases

Protein PTM through the covalent addition or removal of functional groups is a key regulator of protein activity and localization and controls the activation of downstream substrates. It is therefore not surprising that bacterial pathogens have evolved effectors and toxins to chemically modify Rho GTPases. As discussed in more detail below, most PTMs are found in the switch I or switch II loops that regulate interactions with guanine nucleotides (GDP/GTP), GEFs, GAPs, and GTPase effectors.

The large family of clostridial toxins—including the botulinum (C3) toxin (Mohr et al. 1992), as well as the *P. aeruginosa* effectors ExoS and ExoT (Fleiszig et al. 1997)—ADP-ribosylate Rho GTPases. C3 toxins were the first Rho GTPase-modifying enzymes identified and as such were instrumental for distinguishing the roles of different Rho-family isoforms. These toxins and effector proteins ADP-ribosylate RhoA, RhoB, and RhoC on the invariant Asn41 residue found within the nucleotide-binding switch I region (Aktories 2011). The resulting modification enhances the interaction between the Rho GTPase and its GDI, resulting in the sequestration of the GTPase into the cytosol and preventing activation by GEFs (Sehr et al. 1998). Several C3-related toxins, including the Edin toxin of *Staphylococcus aureus* (Wilde et al. 2001) and C3cer in *B. cereus* (Just et al. 1995), have been identified. Interestingly, the insect pathogen *Photorhabdus luminescens* uses a C3-like toxin complex (PTC5) to ADP-ribosylate the switch II region of Rac and Cdc42, preventing GTP hydrolysis and therefore generating a constitutively active GTPase (Lang et al. 2010). Rho GTPases are also activated by the cytotoxic necrotizing factors CNF from *Yersinia pseudo-tuberculosis* and CNF1, CNF2, and CNF3 from *E. coli* and by the *Vibrio* T3SS2 effector VopC (Flatau et al. 1997, Okada et al. 2014, Schmidt et al. 1997). These toxins use their C-terminal domain to catalyze deamination of Gln61 or Gln63 found in the switch II region of Rho GTPases; conversion to a glutamic acid residue prevents GTP hydrolysis, thereby locking the GTPase in an activated site.

Originally described more than 40 years ago (Kingdon et al. 1967), protein modification by addition of adenosine monophosphate (AMP) was recently rediscovered in the *V. parahaemolyticus* effector VopS (Yarbrough et al. 2009) and in *Histophilus somni* IbpA (Zekarias et al. 2010). Both VopS and IbpA proteins contain the FIC (filamentation induced by cAMP) domain, which hydrolyzes the α - β phosphate bond of ATP and transfers AMP to the hydroxyl group of serine, threonine, or tyrosine residues of Rho-family GTPases. AMPylation (or adenylation, as the reaction was originally termed) of Rho GTPases on the switch I region prevents interactions with their downstream effectors, leading to a disorganization of the actin cytoskeleton. It is now clear that FIC-domain proteins can utilize a range of chemical cofactors as donors for PTMs (Roy & Cherfils 2015). The common feature of these cofactors is a diphosphate moiety that can include nucleotides as well as CDP-choline, which is cleaved during the linkage of the adduct to the target protein.

Toxins A and B (also termed TcdA and TcdB, respectively) from *C. difficile*, the lethal and hemorrhagic toxins from *Clostridium sordellii*, the α -toxin from *Clostridium novyi*, and the recently discovered TpeL from *C. perfringens* glycosylate Rho GTPases, leading to their inactivation and their inability to signal to the actin cytoskeleton (Nagahama et al. 2011, von Eichel-Streiber et al. 1996). All these bacterial enzymes target the switch I region, Thr35 in Rac and Cdc42 or Thr37 of Rho isoforms, with either mono-*O*-glycosylation or *N*-acetylglucosamination. Modification at this residue results in a GEF- and GAP-insensitive GTPase (Sehr et al. 1998). Therefore, glycosylated Rho GTPases are unable to participate in nucleotide cycling, resulting in actin cytoskeleton disruption.

ORGANELLE ARCHITECTURE: CONTROLLING MEMBRANE TRANSPORT SYSTEMS BY BACTERIAL PATHOGENS

Membrane-bound organelles are the major feature distinguishing eukaryotic from prokaryotic cells. Such organelles not only sequester chemical reactions but also organize protein and lipid transport systems that support complex cell structures. Like the actin cytoskeleton discussed above, membrane transport systems are heavily targeted by bacterial pathogens. Examples of such systems are the secretory system made up of distinct organelles [including the endoplasmic reticulum (ER), the Golgi apparatus, and the plasma membrane], endolysosomal compartments, and autophagosomes. By specifically manipulating critical aspects of host organelle biogenesis and function, bacteria have the ability to inhibit immune cytokine secretion and immune detection, to establish a replicative vacuole in the host cytoplasm, and/or to avoid destruction through regulation of autophagy, all of which are discussed in more detail below.

Pathogen Targeting the ER-to-Golgi Trafficking Network

The general secretory pathway (GSP), composed of the ER, Golgi apparatus, and cell vesicles, is responsible for processing, sorting, and distributing proteins and lipids throughout the cell. During a host's battle with pathogens, a functional GSP mediates the secretion of cytokines, chemokines, and antimicrobial peptides that are crucial for host defense. Microbial pathogens have therefore developed several strategies to antagonize or exploit the GSP to assist in their infection. It has been known for many years that certain

classes of bacterial toxins traffic along a retrograde pathway, eventually being excreted from the ER compartment (Mukhopadhyay & Linstedt 2013). Although the GSP pathway is clearly hijacked for toxin delivery, other pathogens directly regulate ER and Golgi trafficking networks to avoid immune detection or establish a replicative niche within the host cytoplasm. Here we highlight some recent findings detailing how bacteria regulate the intracellular secretory pathway, especially focusing on the modulation of ARF- and Rab-family GTPases, which are master regulators of organelle structure and ER-to-Golgi vesicle transport.

Golgi Destruction by Coordinated Regulation of ARF and Rab GTPases

GTPases of the ARF and Rab families are master regulators of membrane traffic, organelle structure, and cargo transport (Donaldson & Jackson 2011, Hutagalung & Novick 2011). Like the Rho-family GTPases discussed above, ARFs and Rabs cycle between an inactive GDP-bound form and an active GTP-bound form on the basis of their interactions with GEFs and GAPs. In their active form, ARFs and Rabs associate with ER and Golgi membranes, to which these proteins recruit downstream effectors that promote vesicle fission or membrane docking and fusion events (Cherfils & Zeghouf 2013).

Recent studies have identified bacterial virulence factors that have profound consequences on Golgi structure and function. EspG/VirA-family T3SS effector proteins encoded by EPEC, EHEC, and *Shigella* (Elliott et al. 2001) cause massive Golgi vesiculation when ectopically expressed in host cells (Selyunin et al. 2011) or when secreted by the pathogen (Figure 3*a*) (Dong et al. 2012). Structural studies indicated that EspG specifically binds to the switch I loop and nucleotide-binding pocket of GTP-bound ARFs (Figure 3*b*) (Selyunin et al. 2011). The unique architecture of this interaction prevents GAP-catalyzed GTP hydrolysis, allowing EspG to stabilize active ARF on host membranes (Selyunin et al. 2011). In addition, EspG is a Rab1-specific GAP, thereby targeting two critical components of the host secretory pathway (Dong et al. 2012). Selyunin et al. (2014) described a concerted model for EspG function, highlighting both ARF and Rab interactions (Figure 3*b*). By stabilizing ARF-GTP on Golgi membranes, EspG promotes the recruitment of vesicle tethering factors such as GMAP210 that link incoming vesicles to recipient membranes; simultaneously, EspG prevents vesicle fusion by its Rab1-GAP activity. This concurrent activity of EspG toward ARF and Rab1 is required for EspG to fully inhibit intracellular trafficking and induce the clustered vesicle phenotype observed in cells (Selyunin et al. 2014). Interestingly, *Shigella* VirA exhibits similar Rab1-GAP activity and induces Golgi fragmentation (Dong et al. 2012), yet it does not have the structural elements to support ARF1 binding (Dong et al. 2012, Selyunin et al. 2011). Thus, VirA may coordinate Rab1 inactivation with other signaling components (Selyunin et al. 2014). The Golgi matrix protein GM130 has been identified as a binding partner for EspG in a yeast two-hybrid screen, but whether the EspG-GM130 interaction is linked to EspG-mediated disruption of vesicular transport is unclear (Clements et al. 2011). The molecular study of EspG/VirA function in arresting Golgi trafficking opens new avenues of investigation into the potential roles of these proteins in bacteria pathogenesis. EspG inhibits the secretion of the proinflammatory cytokine IL-8 from HeLa cells during EHEC infection, thus enhancing bacterial intracellular survival (Dong et al. 2012). EspG1 and -G2 mediate tight junction (TJ)

disruption during EPEC infection (Glotfelty & Hecht 2012); the potential of EspG1/G2 to block secretion of TJ proteins may be one undetermined mechanism. For *Shigella*, inactivation of Rab1 by VirA inhibits autophagy-mediated host defense (Dong et al. 2012).

Elimination of *N*-Myristoyl Modification of ARF GTPases

Besides VirA, another *Shigella* T3SS effector protein named IpaJ has recently been implicated in the inhibition of cargo transport through the GSP. According to structure-based bioinformatics analysis, IpaJ exhibits similarities with the C39-like cysteine peptidase family and harbors a classic Cys/His/Asp catalytic triad required for peptide bond cleavage by this family. Substitutions of the three residues abolished IpaJ's ability to induce Golgi fragmentation during *Shigella* infection of host cells, indicating that this function depends on the cysteine protease activity of IpaJ (Burnaevskiy et al. 2013). Further study indicated that IpaJ specifically cleaves the myristoylated N-terminal glycine of host proteins, and one potential target is ARF1, a small GTPase critical for Golgi apparatus structure and function (Burnaevskiy et al. 2013). ARF1 regulates cargo transport through the Golgi apparatus by binding to the Golgi membranes (through *N*-myristoylation) and recruiting the COPI coat complex for vesicle fission (Kahn 2009). IpaJ cleaves the peptide bond between *N*-myristoylated Gly2 and Asn3 of human ARF1, which releases ARF1 from the Golgi membranes and causes fragmentation of the Golgi and disruption of host cargo trafficking (Burnaevskiy et al. 2013).

Profiling the host cell “myristoylome” along with detailed functional studies revealed that IpaJ recognizes and cleaves most *N*-myristoylated proteins in vitro yet exhibits high specificity in vivo, such as targeting ARF and ARL GTPase families in the context of *Shigella* infection (Burnaevskiy et al. 2015). IpaJ recognizes two diverse structural elements on ARF proteins: One element (myristoylated glycine) is common to all myristoylated proteins, whereas the other (the GTPase domain) is unique to GTPase-family proteins (Burnaevskiy et al. 2015). Further work uncovered the ability of IpaJ to inhibit ER exit of STING to antagonize the cGAS/STING cytosolic DNA-sensing pathway involved in the type I interferon response (Dobbs et al. 2015). IpaJ blocks the translocation of STING from ER to ERGIC (ER-Golgi intermediate compartment), which is critical for STING activation. IpaJ also suppresses STING activation of disease-associated STING mutants that causes constitutive ER exit. These functions of IpaJ are dependent on its activity to demyristoylate ARF GTPase (Dobbs et al. 2015).

Hijacking Host ER-to-Golgi Vesicular Transport by Manipulating Rab1 Activity

In addition to blocking the secretory pathway, some pathogens exploit the trafficking system to establish intracellular persistence. *Legionella* is an intracellular pathogen that hijacks ER-derived vesicles to create a *Legionella*-containing vacuole (LCV) for replication. The biogenesis and maturation of an LCV involve rewiring the destination of secretory vesicles derived from the ER; such rewiring is promoted by acquisition of Rab1 GTPase. The molecular mechanisms underlying exploitation of host cell Rab1 by *Legionella* have been well studied and involve both regulation of the Rab1 guanine nucleotide activation cycle and chemical modifications by bacterial enzymes (Figure 4a). The SidM/DrrA effector, which possesses both GDI displacement factor activity and GEF activity, promotes the recruitment

of Rab1 to the LCV (Brombacher et al. 2009, Machner & Isberg 2007). Because Rab1 can rapidly undergo GAP-mediated hydrolysis, SidM/DrrA further stabilizes GTP-active Rab1 on the LCV by AMPylating at Tyr77 of the switch loop. This PTM blocks access of GAPs to the nucleotide-binding pocket, thereby stabilizing membrane-bound GTP-Rab1 (Muller et al. 2010). Importantly, this chemical modification can be reversed by the deAMPylating enzyme SidD (Neunuebel et al. 2011, Tan & Luo 2011), and subsequently Rab1 can be inactivated by the *Legionella* GAP LepB (Schuerch et al. 2005). Ultimately, the regulation of Rab1 by both GEFs/GAPs and AMPylation controls the timing and location of Rab1 signal transduction from the LCV. Additional *Legionella* enzymes targeting Rab1 include AnkX and LegA8, which catalyze the covalent attachment of CDP-choline to Rab1 (phosphocholination); Lem3 can reverse this process (Goody et al. 2012, Tan et al. 2011). In addition, the effector SidC possesses ubiquitin ligase activity that is correlated with Rab1 ubiquitination, yet direct ubiquitination of Rab1 by SidC has not been shown (Horenkamp et al. 2014, Hsu et al. 2014). Thus, the ubiquitination of Rab1 is likely mediated through an indirect unknown mechanism. Crystal structure analysis suggests that C-terminal Rab1 ubiquitination may interfere with Rab1-GDI interaction, but the exact function of this modification in *Legionella* infection requires further investigation (Horenkamp et al. 2014).

Pathogen Remodeling of the Endolysosomal System

Much like *Legionella's* interaction with the host secretory pathway, many bacteria have evolved strategies to remodel the host endolysosomal system to create a replication-competent vacuole. Although the individual virulence factors may be different among bacterial species, these pathogens have evolved common strategies to modify the vacuole to form a degradation-resistant and replication-permissive microenvironment. Many pathogens deliver effector proteins to target host cell proteins or lipids to modify the vacuolar membrane composition or promote selective interactions with the endolysosomal system, which prevents the pathogens from degradation by lysosomes, yet allows interactions with host organelles that are required for both vacuole expansion and nutrient uptake.

Salmonella is perhaps the best-studied pathogen that exploits the host endolysosomal system. Immediately following internalization, these bacteria establish a *Salmonella*-containing vacuole (SCV) that provides three major advantages. First, the manipulation of the vacuole components restricts the fusion of the vacuole with the host lysosome. Second, the vacuole slows replication with the host, thereby providing a stable niche to persist in the host cell environment while avoiding cytosolic immune receptors. Finally, the SCV is trafficked to a perinuclear region of the cell, from where it acquires nutrients from its environment. This process is mediated predominantly by effector proteins secreted by the SPI-2 T3SS, although several SPI-1 effectors link invasion to SCV biogenesis (Figure 4*b*). For example, the phosphoinositide phosphatase SopB, which is required for actin-mediated *Salmonella* internalization, decreases the negative surface charge of the vacuolar membrane shortly after invasion (Bakowski et al. 2010). This event leads to the dissociation of several trafficking proteins from the vacuole, helping to prevent lysosome fusion. The SCV then migrates to the perinuclear region in close proximity to the Golgi network. A main feature of SCV maturation is the formation of *Salmonella*-induced filaments (SIFs) that extend from the vacuole at late stages of infection.

A key effector protein for SCV maintenance and SIF formation is SifA (Beuzon et al. 2000). Shortly after secretion, SifA is targeted via C-terminal acylation to the SCV, from where SifA interacts with two host proteins: SKIP (SifA kinesin-interacting protein) and RhoA GTPase. The interaction with SKIP has two seemingly opposing roles in SCV biogenesis. First, SKIP was originally shown to inhibit plus end-directed microtubule kinesin-1 activity at the SCV, thereby restricting microtubule motility of the SCV toward the cell periphery and maintaining its position in a perinuclear region permissive for *Salmonella* replication (Boucrot et al. 2005). Second, SifA recruitment of SKIP to the SCV is required for SIF formation, which maintains SCV integrity and is a kinesin-1-dependent process. Although the order of events required for SIF formation remains obscure, several additional effector proteins, including PipB2, coordinate kinesin-1 recruitment to SIFs (Henry et al. 2006). It has been suggested that SifA recruits inactive kinesin-1 to membranes and that PipB2 activates the motor protein to generate SIF protrusions from the vacuole. In addition, the SifA/SKIP complex sequesters Rab9 and subverts Rab9-dependent retrograde trafficking of mannose-6-phosphate receptors, thereby attenuating lysosome function (McGourty et al. 2012). Two recent reports also identified PLEKHM1 as a new host target for SifA. PLEKHM1 localizes to distinct regions of the SCV, where it interacts directly with both Rab7 and SifA to regulate membrane biogenesis of the SCV and maintain the normal morphology of this compartment (McEwan et al. 2015a,b).

Another SPI-2 T3SS effector, SseJ, acts complementarily with SifA to regulate dynamics of the SCV membrane. Unlike *sifA*⁻ *Salmonella* strains that escape the SCV, *sifA*⁻/*sseJ*⁻ double-mutant strains do not lose vacuole integrity during infection (Ruiz-Albert et al. 2002). In addition, ectopic expression of SifA and SseJ in HeLa cells induces endosomal tubulation, similar to the case for SIFs that form during *Salmonella* infection. This endosomal tubulation is also observed when SseJ is coexpressed with GTP-bound RhoA, RhoB, and RhoC, and SifA likely activates RhoA-family GTPases that coordinate SseJ activity (Christen et al. 2009). Structural studies indicated that, in addition to binding SKIP, SifA belongs to the WxxxE family of Rho GEFs. It has therefore been proposed that SseJ, SifA, SKIP, and RhoA constitute a complex that promotes vacuole tubulation as a precursor to SIF formation (Ohlson et al. 2008). SseJ may also modulate the composition of cholesterol on SCV membranes by acting as a RhoA-dependent glycerophospholipid-cholesterol acyltransferase enzyme (Christen et al. 2009, Lossi et al. 2008, Nawabi et al. 2008). Besides SseJ, additional SPI-2 effectors, including SopD2, SpvB, SteA, SseF, and SseG, are involved in SIF formation (Birmingham et al. 2005; Domingues et al. 2014; Knodler & Steele-Mortimer 2005; Krieger et al. 2014; Schroeder et al. 2010, 2011). SseF and SseG are also implicated in the perinuclear positioning of SCVs by recruiting dynein (Birmingham et al. 2005, Deiwick et al. 2006, Kuhle et al. 2006, Salcedo & Holden 2003). As work over the past decade has indicated, SCV positioning and maintenance are highly complex processes requiring the concerted action of both bacterial effector proteins and host substrates.

Like *Salmonella*, *Brucella* has developed several strategies to restrict fusion of the *Brucella*-containing vacuole (BCV) with lysosome compartments through the function of the T4SS effector VirB (Comerci et al. 2001, de Figueiredo et al. 2015). *Legionella* delivers multiple effectors to escape phagolysosomal killing, as well as effector-independent mechanisms

(Prashar & Terebiznik 2015). Strikingly different from other vacuolar pathogens that avoid fusion of the vacuole with the lysosome, the *Coxiella burnetii*-containing vacuole is loaded with lysosome enzymes. *C. burnetii* is unique in its ability to survive in this harsh environment; it may exploit the acidic pH of the lysosome-like vacuole for metabolic activation (Ghigo et al. 2012, van Schaik et al. 2013).

Interference of Autophagy Signaling

Autophagy can be a host defense mechanism to target intracellular pathogens for lysosomal degradation. Although some bacteria are targeted and eventually destroyed by autophagy, several pathogens have developed strategies to avoid autophagy or even hijack the host's autophagic machinery for intracellular growth and survival.

Inhibition and evasion of autophagy—Several virulence factors have evolved to disrupt autophagosome formation. For example, the Eis (enhanced intracellular survival) protein secreted by *Mycobacterium tuberculosis* inhibits JNK-dependent autophagy activation (Kim et al. 2012, Shin et al. 2010). Rab1 inactivation by VirA suppresses host autophagy signaling (Dong et al. 2012) because Rab1 also plays a role in autophagosome formation (Huang et al. 2011). *Listeria* phospholipases PlcA and -B inhibit autophagic flux and autophagosome formation, preventing efficient targeting of cytosolic bacteria by autophagy machinery (Tattoli et al. 2013). *Legionella* delivers an effector protein, RavZ, which is a cysteine protease to deconjugate Atg8/LC3 from phosphatidylethanolamine and inhibit autophagosome formation (Choy et al. 2012). Additionally, *Salmonella* can escape from autophagy-mediated degradation by promoting mTOR reactivation on the surface of SCV, although the bacterial factors and mechanisms involved in this process remain unknown (Kim et al. 2012). Finally, some pathogens like *Bacillus anthracis* and *V. cholerae* express toxins that inhibit autophagy induction by increasing cyclic AMP levels (Shahnazari et al. 2011).

Pathogens can also avoid autophagic degradation by blocking autophagosome maturation. *M. marinum* recruits LC3 to phagosomes and can regulate the fate of these phagosomes. These LC3-decorated phagosomes do not acquire lysosomal enzymes, indicating that the infecting bacteria block autophagosomal maturation (Lerena & Colombo 2011). Similarly, *Helicobacter pylori* (Raju et al. 2012), *Yersinia pestis* (Pujol et al. 2009), *Chlamydia trachomatis* (Al-Younes et al. 2004, Yasir et al. 2011), and *Porphyromonas gingivalis* (Dorn et al. 2001) disrupt autophagosomal maturation by blocking autophagosome acidification or lysosomal degradation, although the molecular mechanisms have not been elucidated.

Certain pathogens have evolved the ability to mask themselves from autophagy recognition. For example, when *Listeria* enters the cytosol, its surface protein ActA recruits host proteins of the actin polymerization machinery (such as actin, Arp2/3, and VASP) to polymerize actin and form actin tails at the bacterial surface. A higher proportion of *actA* mutant bacteria are sequestered in LC3-positive vacuoles than are wild-type bacteria (Birmingham et al. 2007, Yoshikawa et al. 2009). *Listeria* also uses the virulence factor InlK to recruit a host protein named major vault protein (MVP) to decorate its surface for autophagy evasion, and this new strategy to avoid autophagy is independent of ActA (Dortet et al. 2011, Neves

et al. 2013). *Shigella* also counters autophagic attack through effector protein IcsB. IcsB competes with host ATG5 for binding to the bacterial surface protein IcsA, thereby masking the bacteria from autophagy recognition (Ogawa et al. 2005). IcsB may also prevent *Shigella* from autophagic targeting by masking the bacteria from septin binding (Mostowy et al. 2010). A recent study has shown that *Salmonella* utilizes a deubiquitinase, SseL, to mask the presence of ubiquitinated aggregates formed during the infection of epithelial cells or macrophages and to reduce the recruitment of autophagic components (Mesquita et al. 2012, Thomas et al. 2012). *Francisella* may avoid autophagy recognition by using the surface polysaccharidic O-antigen through an as-yet-unidentified mechanism (Case et al. 2014).

Exploitation of autophagy—In some cases, bacteria even exploit the host's autophagic machinery to benefit their own infection. For example, *Coxiella* replicates in a vacuole decorated by autophagic markers LC3, Beclin 1, and RAB24 (Gutierrez et al. 2005, Vazquez & Colombo 2010). Induction of autophagy promotes the infectivity of *Coxiella*, and blocking of autophagy impairs bacterial replication. Autophagic proteins play important roles in the maturation of the vacuole that supports *Coxiella* replication (Beron et al. 2002, Gutierrez et al. 2005). Infection of *Coxiella* actively modulates the autophagic pathway, including steps such as LC3 processing and Beclin 1 recruitment to the vacuole, although the bacterial factors involved in these processes remain to be further elucidated (Romano et al. 2007, Vazquez & Colombo 2010). *Coxiella* actively recruits and fuses autophagosomes to the vacuole to deliver nutrients for pathogen replication; the bacterial T4SS and effector Cig2 is required for this process (Newton et al. 2014, Winchell et al. 2014).

Different from pathogens that exploit autophagy to acquire host cell nutrients and support their own replication, *Brucella* subverts autophagy machinery to promote cell-to-cell spreading. At a late stage of infection, the replicative *Brucella*-containing vacuole is converted into a compartment with autophagic features, the autophagic *Brucella*-containing vacuole (aBCV). This conversion requires the activity of proteins that initiate autophagy, including ULK1, Beclin 1, ATG14L, and PI3K, but is independent of autophagy elongation factors ATG5, ATG16L1, ATG4B, ATG7, and LC3B. BCV formation does not contribute to bacterial proliferation but promotes bacterial cell-to-cell spread. Further studies are needed to clarify the mechanisms by which aBCV mediates bacterial release and reinfection (Starr et al. 2012).

V. parahaemolyticus is the first extracellular pathogen characterized to exploit autophagy for its own benefit. Two *V. parahaemolyticus* T3SS1 effectors, Vp1659 (Zhou et al. 2010) and VopQ, and one T6SS effector, VgrG2 (Yu et al. 2015), have been found to be involved in the induction of autophagy. VopQ induces acute autophagy, causes the infected cell to digest itself, and prevents phagocytosis of the bacteria (Burdette et al. 2008, 2009). Molecularly, VopQ alters autophagic flux by binding to the V-ATPase and disrupting host lysosome ion homeostasis (Sreelatha et al. 2013). VopQ-mediated autophagy also interferes with the NLRC4 inflammasome, thus contributing to bacteria evasion from inflammasome-mediated host immune responses (Higa et al. 2013).

OUTLOOK

This article details individual effector proteins and toxins that target animal cell structure and function. The virulence factors described often exhibit novel enzymatic mechanisms that alter actin cytoskeleton dynamics and membrane architecture. Importantly, progress in elucidating effector/toxin activities has required reductionist approaches and isolated biochemical or cell systems. As pathogens rely on the synchronized activities of multiple effectors, studying the mechanisms by which each effector functions individually paints an incomplete picture of the infection processes. New experimental approaches are needed to clarify the location and timing of effector activity, which will begin to elucidate how bacteria hijack signaling systems in a coordinated manner. The future understanding of these complex host-pathogen interactions will require more holistic approaches that bridge the gap between reconstitution studies in vitro, cell biology models of infection, and animal models of pathogenesis. Prior to achieving this goal, however, it remains important to elucidate the structure and enzymatic mechanisms of poorly characterized effectors and toxins, as bacterial infection is the sum of all parts of the system. Finally, the study of toxins and effectors that alter cell architecture provides significant insight into fundamental biological processes and holds great promise to reveal new leads for drug discovery and biotechnology.

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Glossary

Globular actin (G-actin)

monomeric actin

Filamentous actin (F-actin)

composed of a double-stranded helical polymer of actin subunits

Nucleation-promoting factors (NPFs)

proteins (e.g., N-WASP/WAVE) that activate actin nucleators such as the Arp2/3 complex or formins

Arp2/3 complex

a seven-subunit protein complex that creates branched actin networks by nucleating actin polymerization from the sides of existing actin filaments

Actin-based motility

intracellular pathogens that hijack actin polymerization machinery for movement in and between cells

Formins

actin nucleators composed of formin homology (FH2) domains that polymerize actin at the plus end of a filament

Wasp homology 2 (WH2) domains

18-amino-acid sequences that bind to actin monomers and profilin and aid polymerization

SPI-1 and SPI-2

Salmonella encodes two T3SSs; SPI-1 secretes effectors required for invasion, and SPI-2 secretes effectors required for SCV biogenesis

ADF/cofilin

an actin-binding protein that disassembles actin filaments

Gelsolin

an actin filament-severing protein

ADP-ribosylation

the addition of ADP-ribose to target proteins

Nicotinamide adenine dinucleotide (NAD)

the NAD⁺ cofactor is required for ADP-ribosylation

CaaX motif

an amino acid sequence found at the C terminus of proteins that is prenylated and is required for membrane anchoring of Ras-family GTPases

Tethering factors

structural proteins such as GMAP210 and GM130 that tether vesicles to organelles or maintain organelle integrity

Myristoylation

the addition of a 14-carbon saturated fatty acid to the N-terminal glycine of proteins

STING

an ER resident protein that is activated by cyclic dinucleotides and signals to activate the interferon response

***Legionella*-containing vacuole (LCV)**

exists in a cell compartment between the ER and Golgi and exhibits properties of ER membranes

Phospholipase

an enzyme that hydrolyzes phospholipids to produce fatty acids and molecules often used in signal transduction

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BACTERIA CONVERTING ACTIN INTO PATHOGENIC SECOND MESSENGERS

Because actin is one of the most abundant proteins in cells, it has been unclear how low concentrations of toxins or effector proteins delivered into host cytoplasm may have such a robust effect on cell architecture and function. Exciting new studies suggest that actin can be converted into a second messenger that amplifies low abundant toxin function (Heisler et al. 2015). The actin cross-linking domain (ACD) found in toxins such as the multifunctional autoprocessing repeats-in-toxin (MARTX)-containing toxins covalently cross-links actin into oligomers that are structurally incompatible for polymerization, resulting in rapid cell rounding with loss of all polymerized actin (Fullner & Mekalanos 2000, Lin et al. 1999). It was previously thought that ACD toxicity was a consequence of cross-linking the G-actin pool into polymerization-deficient oligomers (Cordero et al. 2006, Fullner & Mekalanos 2000, Lin et al. 1999). However, due to the inconsistencies between in vitro ACD activity (which is low) and cell G-actin concentrations (which are high), Heisler et al. (2015) proposed that toxicity is caused by ACD cross-linked actin oligomers targeting upstream actin regulator proteins. Indeed, they found that actin oligomers poison the formin family of actin nucleators. Therefore, bacterial toxins not only can target existing signaling networks but also can amplify pathogenic signaling cascades by generating novel toxic second messengers.

PATHOGENS TRIGGERING HOST IMMUNITY BY REGULATION OF THE ACTIN CYTOSKELETON

Receptors that recognize conserved microbial-associated molecular patterns (MAMPs) carry out pathogen detection. As many of the classical MAMPs, such as peptidoglycan and LPS, are shared among commensals and pathogens, the mere presence of the bacterial product is not indicative of a pathogenic infection. To distinguish friend from foe, immune receptors monitor the cytosol for evidence of bacterial pathogenic activity. Recent studies suggest that the innate immune system monitors bacterial regulation of Rho GTPase activity. For example, the *Salmonella* GEF SopE induces NF- κ B activation in a NOD1-dependent manner (Keestra et al. 2013). Normally, NOD1 and NOD2 recognize the presence of peptidoglycan in the cytosol (Keestra & Baumlér 2014, Tattoli et al. 2007). However, excessive activation of Rho GTPases during *Salmonella* invasion may be the signal for NOD1 activation during infection. Furthermore, the Pyrin inflammasome is activated by chemical modifications of the switch I loop in Rho GTPase isoforms (Xu et al. 2014). These modifications include glycosylation by the large clostridial TcdA/B toxins, AMPylation by FIC domain–encoding effectors, ADP-ribosylation by the C2 or C3 toxin, and deamidation by an unidentified *Burkholderia cenocepacia* T6SS effector (Xu et al. 2014). Future work will likely reveal the mechanism behind NOD1 and Pyrin activation via Rho GTPase regulation.

Figure 1.

Regulation of actin dynamics by bacterial toxins and effector proteins. Bacterial pathogens have evolved multiple strategies to both positively and negatively regulate actin dynamics. Actin polymerization is inhibited through ADP-ribosylation of G-actin into nonpolymerizable monomers by the C2 toxin, the iota toxin, the VIP (vegetative insecticidal protein) toxin, and the *Salmonella* effector SpvB. ACD (actin cross-linking domain) toxin-mediated actin cross-linking poisons the formin family of actin nucleators. Actin polymerization is inhibited by bacterial effectors through the removal of membrane-bound Rho GTPases via either sequestration of the lipid group by YopO/YpkA or proteolytic cleavage of the lipid group from Rho GTPases by YopT. Additionally, inactivation of Rho GTPases via effector GAP (GTPase-activating protein) activity of SptP and ExoT shuts off actin polymerization. Rho GTPases undergo posttranslational modification (PTM) by ADP-ribosylation via C3 toxins and effectors ExoS and ExoT and by AMPylation by VopS and IbpA, preventing Rho GTPase activation of actin polymerization. Bacterial pathogens have also evolved mechanisms to induce actin dynamics through various mechanisms. VopL/F, Sca2, and SipC directly nucleate actin. Additionally, VopV, SipC, and SipA stabilize actin filaments by cross-linking. Bacterial effectors directly activate or function as nucleation-promoting factors to promote Arp2/3-mediated actin nucleation. Activation of Rho GTPase signaling by SopE- and WxxxE-family members of bacterial GEFs (guanine nucleotide exchange factors) also induces actin polymerization.

Figure 2.

Bacterial effectors mimic eukaryotic actin-binding proteins and regulators. Bacterial effector proteins can structurally or functionally mimic host proteins. (a) The *Rickettsia* actin nucleator Sca2 (PDB: 4J7O) is a structural mimic of eukaryotic formin Bni1p (PDB: 1Y64) (Otomo et al. 2005). Formins adopt a ringlike structure that aids in barbed-end polymerization. (b) Several classes of bacterial guanine nucleotide exchange factors (GEFs), including SopE- and WxxxE-family members (*Escherichia coli* Map shown here; PDB: 3GCG), functionally mimic eukaryotic GEFs (Vav1 shown here; PDB: 3KY9). Both bacterial and eukaryotic GEFs of the Dbl family induce similar structural rearrangements in Rho GTPase proteins, but they do so using completely distinct structural architectures and catalytic residues. In addition, unlike eukaryotic GEFs, which are highly decorated with various accessory domains, bacterial GEFs are compact and encode minimal accessory domains. Abbreviations: DH, Dbl homology domain; PH, pleckstrin homology domain.

Figure 3. Bacterial effector regulation of organelle structure and function. (a) As shown by correlative light electron microscopy, the Golgi apparatus vesiculates shortly after microinjection of the *Escherichia coli* effector EspG. A normal Golgi apparatus is shown at the right. (b) The structure of EspG in complex with Rab1 and ARF1 has provided important insights into the mechanism of EspG-mediated Golgi vesiculation (PDB: 4FME).

Figure 4.

Effector-mediated remodeling of pathogen-containing vacuoles: *Legionella*-containing vacuoles (LCV) and *Salmonella*-containing vacuoles (SCV). (a) *Legionella* encodes multiple effectors that regulate Rab1 activity. SidM is a multidomain effector with both Rab1 GEF (guanine nucleotide exchange factor) activity and AMPylation activity (black circle labeled A). SidD can deAMPylate Rab1. Rab1 can be inactivated by the GAP (GTPase-activating protein) LepB. Furthermore, Rab1 can be modified by the addition of phosphocholine by AnkX through its FIC (filamentation induced by cAMP) domain (black circle labeled C) or by the removal of the phosphocholine by Lem3. Lastly, the ubiquitin ligase SidC is thought to ubiquitinate (black circle labeled Ub) Rab1, preventing its interaction with its GDI (guanine nucleotide dissociation inhibitor). (b) *Salmonella* modifies its SCV through the action of multiple effectors. The SPI-1-secreted effector SopB, a phosphoinositide phosphatase, is involved in invasion and alters the surface charge (black circles labeled with minus signs) of the SCV to prevent lysosomal fusion. SifA is a key effector regulating SCV maintenance and *Salmonella*-induced filament (SIF) formation. SifA, a SPI-2 effector, interacts with the host protein SKIP (SifA kinesin-interacting protein) to retain SCV perinuclear positioning through kinesin and PipB2 interactions. In addition, the GEF domain of SifA is thought to activate RhoA GTPase at the SCV, which subsequently induces the acyltransferase activity of the SPI-2 effector SseJ, generating cholesterol esters on the SCV (black circle labeled CE). These events are thought to promote SIF formation. SseL, through its deubiquitinase (DUB) activity, reverses SCV ubiquitination and host cell recognition by autophagosomes.