How to rewire the host cell: A home improvement guide for intracellular bacteria

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Intracellular bacterial pathogens have developed versatile strategies to generate niches inside the eukaryotic cells that allow them to survive and proliferate. Making a home inside the host offers many advantages; however, intracellular bacteria must also overcome many challenges, such as disarming innate immune signaling and accessing host nutrient supplies. Gaining entry into the cell and avoiding degradation is only the beginning of a successful intracellular lifestyle. To establish these replicative niches, intracellular pathogens secrete various virulence proteins, called effectors, to manipulate host cell signaling pathways and subvert host defense mechanisms. Many effectors mimic host enzymes, whereas others perform entirely novel enzymatic functions. A large volume of work has been done to understand how intracellular bacteria manipulate membrane trafficking pathways. In this review, we focus on how intracellular bacterial pathogens target innate immune signaling, the unfolded protein response, autophagy, and cellular metabolism and exploit these pathways to their advantage. We also discuss how bacterial pathogens can alter host gene expression by directly modifying histones or hijacking the ubiquitination machinery to take control of several host signaling pathways.

Introduction

Invading intracellular bacteria have to continuously battle with the host for survival. Therefore, it is not surprising that most bacterial pathogens have evolved fascinating mechanisms to subvert host cell defense mechanisms or exploit its nutrient inventory. To do so, bacterial pathogens are armed with an arsenal of different virulence factors, called effectors, which can specifically manipulate cellular pathways to their advantage. As soon as a bacterial pathogen enters the host cell, the host tries to degrade the bacteria in the lysosome; thus, bacterial pathogens need to prevent, delay, or escape contact with lysosomes (Luzio et al., 2007). In addition, the pathogen encounters innate immune signaling, which leads to a proinflammatory cytokine response. Furthermore, intracellular bacteria must defend against autophagic clearance and activation of homeostatic pathways, such as the unfolded protein response (UPR), that can lead to apoptosis (Cemma and Brumell, 2012; Celli and Tsolis, 2015). Finally, the pathogen needs to build itself a replicative niche where it can acquire nutrients from the host; for many intracellular pathogens, this niche is a subcellular membrane-bound compartment that is conducive to its replication (Kumar and Valdivia, 2009). How pathogens subvert membrane transport pathways has been extensively studied (Alix et al., 2011; Asrat et al., 2014a); therefore, in this review, we will focus on pathogenic strategies that subvert key host defense mechanisms and manipulate host signaling pathways to create a suitable intracellular niche. The innate immune response, the UPR pathway, and autophagy are central to host defense. However, they are also homeostatic pathways that can be exploited by intracellular pathogens. Likewise, the means of transcriptional and posttranslational regulation of these pathways through histone modifications and ubiquitination can also be co-opted by pathogens to manipulate the cell signaling pathways and gene expression of the host.

Sensing of bacterial colonization by the innate immune system

Innate immune cells have the remarkable ability to sense bacteria, both extracellularly and intracellularly, and mount an appropriate immune response that matches the level of threat (Akira et al., 2006). Pathogen recognition receptors (PRRs) recognize broadly conserved pathogen-associated molecular patterns (PAMPs) and trigger multiple signaling pathways, which ultimately lead to changes in gene expression of proinflammatory cytokines and genes that regulate antimicrobial processes (Akira et al., 2006). In addition, PRRs in the cytosol control potent antimicrobial responses including the inflammasome,

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Abbreviations used: ATF, activating transcription factor; CCV, *Coxiella*-containing vacuole; cGAMP, 2′3′-cyclic guanosine monophosphate–adenosine monophosphate; cGAS, cyclic GMP-AMP synthase; CHOP, C/EBP homologous protein; CSP, cytosolic surveillance pathway; DUB, deubiquitinase; ERK, extracellular signal–regulated kinase; IκB, inhibitor of κB; IKK, IκB kinase; Lgt, *Legionella* glucosyltransferase; LLO, listeriolysin O; Mtb, *Mycobacteria tuberculosis*; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor-κB; NLR, NOD–like receptor; PAMP, pathogen-associated molecular pattern; PE, phosphatidyl-ethanolamine; PERK, PKR-like ER kinase; PI3P, phosphatidylinositol 3-phosphate; PRR, pathogen recognition receptor; ROS, reactive oxygen species; T4SS, type IV secretion system; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; UPR, unfolded protein response.

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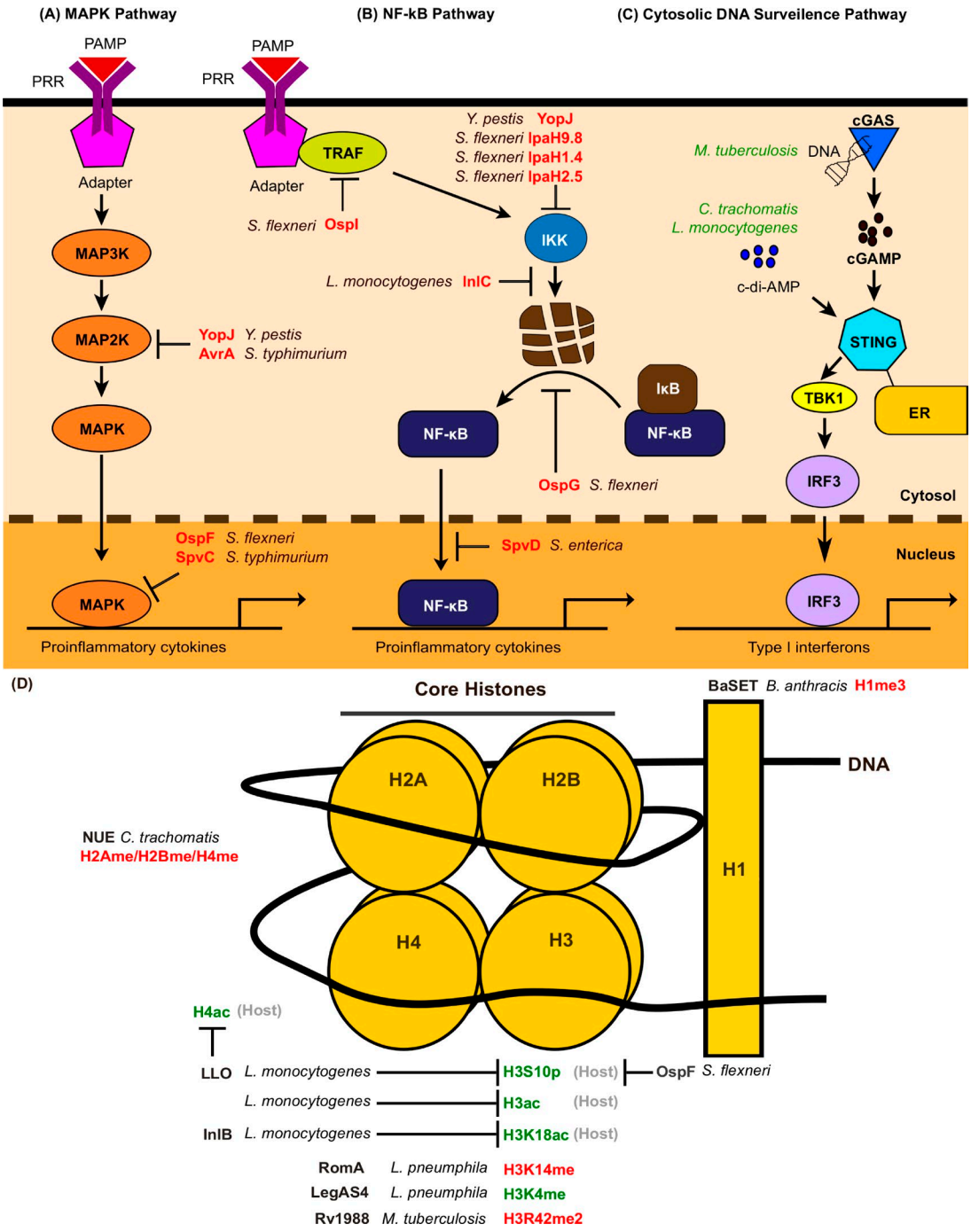

Figure 1. **Modulation of innate immunity signaling pathways by bacterial pathogens.** (A) Stimulation of PRRs by PAMPs activates a MAPK signaling cascade. (B) PRR activation also releases NF-κB from its inhibitor, IκB, which allows NF-κB to translocate to the nucleus and induce the expression of proinflammatory cytokines. (C) STING, a major regulator of the CSP that is anchored to the ER, is activated by cyclic dinucleotides cGAMP produced by DNA sensing from cGAS as well as secreted c-di-AMP produced by bacteria. Activation of the STING/TBK1/IRF3 pathway leads to a type I IFN response. (D) Posttranslational modifications to histones by bacterial pathogens. Bacterial effectors can inhibit host-mediated histone modifications by indirectly causing the reversal of these modifications. Bacterial effectors can also directly modify histones. H, histones; red, inhibitory histone modifications; green, activating histone modifications; black, pathogen effector protein; italics, pathogen. Histone modifications: phosphorylation (p), acetylation (ac), methylation (me), dimethylation (me2), and trimethylation (me3). Bacteria or secreted bacterial effectors can either inhibit (red) or activate (green) these innate immune signaling pathways.

autophagy, and the cytosolic surveillance pathway (CSP; Deretic and Levine, 2009; Lamkanfi and Dixit, 2011; Radoshevich and Dussurget, 2016). Finally, the proinflammatory cytokine response that is initiated by infected cells can also activate neighboring, uninfected bystander cells to mount a multicellular immune response to the threat (Holmgren et al., 2017). Accordingly, successful bacterial pathogens attempt to usurp host innate immunity at all levels of defense (Reddick and Alto, 2014). Sensing the disruption to the cellular homeostasis inflicted by bacterial effector proteins allows the host to

discriminate between pathogens and nonpathogens (Vance et al., 2009). Pathogen recognition by the innate immune system has been extensively reviewed (Akira et al., 2006; Mogensen, 2009; Vance et al., 2009); here, we will highlight more recent examples of modulation of innate immune signaling pathways by intracellular bacterial pathogens.

Innate immune signaling through PRRs. Host defense against bacterial pathogens greatly relies on PRRs that recognize specific PAMPs such as nucleic acids, cell wall components, and proteins from fungi, bacteria, viruses, and parasites. Two major PRR classes are Toll-like receptors (TLRs) and NOD-like receptors (NLRs), which collectively recognize PAMPs at the cell surface as well as in the cytoplasm (Mogensen, 2009). PAMP recognition by PRRs activates a proinflammatory response via two major signaling pathways that are mediated by MAPKs and nuclear factor-κB (NF-κB), respectively (Arthur and Ley, 2013; Fig. 1, A and B). Activated TLRs and NLRs associate with specific adapter proteins and initiate MAPK signaling via downstream MAP3K, MAP2K, and MAPK phosphorylation cascades (Fig. 1 A). Three major MAPK families mediate pro-survival signaling pathways: extracellular signal-regulated kinases (ERKs), p38, and JNK. Stimulated PRRs at the cell surface and in the cytoplasm activate these MAPKs, which in turn activate cytoplasmic transcription factors that induce the expression of proinflammatory cytokines in the nucleus (Arthur and Ley, 2013; Fig. 1 A).

In addition to MAPK signaling, NF-κB is essential in regulating the innate immune response of the host and is activated downstream of most PRRs (Dev et al., 2010; Fig. 1 B). In the absence of infection, NF-κB is associated with the inhibitor of κB (IκB) in the cytoplasm. Activated PRRs recruit adapter proteins, such as TNF receptor–associated factors (TRAFs), which activate IκB kinase protein complex (IKK). IKK phosphorylates IκB, which is subsequently ubiquitinated and proteosomally degraded. Released NF-κB is then able to enter the nucleus and induce the expression of proinflammatory proteins (e.g., TNF and IL-6; Hayden and Ghosh, 2008; Fig. 1 A).

A third major host defense against intracellular bacterial pathogens is the CSP, which recognizes hallmarks of infection, such as DNA in the cytoplasm, and induces a type I IFN response (O'Riordan et al., 2002; Fig. 1 C). Stimulator of interferon genes (STING) is a major player in the CSP (Ishikawa and Barber, 2008). STING activates TANK-binding kinase 1 (TBK1), which phosphorylates IFN regulatory factor 3 (IRF3) and induces IFN expression (Radoshevich and Dussurget, 2016). The cytosolic DNA sensor, cyclic GMP-AMP synthase (cGAS), can activate STING by producing the second messenger, 2′3′-cyclic guanosine monophosphate–adenosine monophosphate (cGAMP; Sun et al., 2013). Cyclic dinucleotides (CDNs) produced by bacteria can also activate the STING/TBK1/IRF3 pathway and induce an IFN response in the host independently of cGAS (Burdette et al., 2011; Sauer et al., 2011; Fig. 1 C).

The diversity of PRRs, PRR ligands, and PRR adapter proteins allows for a specific and highly regulated innate immune response to pathogen invasion. The target, degree, and timing of gene expression are finely tuned to the specific PRR–PAMP interactions, which activate different subsets of transcription factors (Dev et al., 2010). More importantly, MAPK-, NF-κB–, and CSP-mediated transcriptional programs can synergize upon activation of distinct PRRs to mount an inflammatory response that is appropriate for a given pathogen (Akira et al., 2006).

Cytosolic sensors of intracellular pathogens. Three recent studies identified cGAS as a major host sensor of *Mycobacteria tuberculosis* (Mtb) DNA that is responsible for inducing a robust IFN response in the host during Mtb infection (Collins et al., 2015; Wassermann et al., 2015; Watson et al., 2015; Fig. 1 C). The IFN response induced by wild-type Mtb is cGAS-dependent and activates IRF3 through the STING/TBK1/IRF3 signaling pathway (Manzanillo et al., 2012; Collins et al., 2015; Wassermann et al., 2015; Watson et al., 2015). The ESX-1 secretion system of Mtb is required to produce an

Table 1. **Glossary of effectors: Bacterial modulation of innate immunity**

Bacteria	Effector	Host target	Target pathways	Outcome	Mode of action	Reference
L. pneumophila	Lgt1, Lgt2, Lgt3	eEF1A	Protein translation	Inhibition of protein translation; enhanced immune response	Glucosyltransferase	Belyi et al., 2006, 2008; Fontana et al., 2011, 2012
C. difficile[a]	TcdA	Unknown	Unknown	Enhanced immune response	Glucosyltransferase	Cowardin et al., 2016
P. aeruginosa[a]	ToxA	Unknown	Protein translation	Enhanced immune response	AB exotoxin	Dunbar et al., 2012; McEwan et al., 2012
Y. pestis[a]	YopJ	MAP2Ks	MAPK	Inhibits MAPK and NF-κB signaling	Serine/threonine acetyltransferase	Orth et al., 1999
		IKK	NF-κB			Mukherjee et al., 2006
		TAK1 (MAP3K)	NF-κB			Paquette et al., 2012
S. typhimurium	AvrA	MAP2Ks	MAPK	Inhibits apoptosis	Serine/threonine acetyltransferase	Jones et al., 2008; Wu et al., 2012
L. monocytogenes	InlC	IKK	NF-κB	Reduces degradation of IκB	Binds IKK	Gouin et al., 2010
S. flexneri	IpaH1.4	LUBAC	NF-κB	Inhibits the activation of IKK	E3 ubiquitin ligase	de Jong et al., 2016
S. flexneri	IpaH2.5	LUBAC	NF-κB	Inhibits the activation of IKK	E3 ubiquitin ligase	de Jong et al., 2016
S. flexneri	IpaH9.8	IKK	NF-κB	Proteasomal degradation of IKK	E3 ubiquitin ligase	Ashida et al., 2010
S. flexneri	OspG	E2 ubiquitin-conjugating enzyme	NF-κB	Inhibits the degradation of IκB	Kinase	Kim et al., 2005; Zhou et al., 2013; Pruneda et al., 2014
S. flexneri	OspI	Ubc13 (E2 ubiquitin-conjugating enzyme)	NF-κB	Inactivates Ubc13 to prevent the activation of TRAF6	Glutamine deamidase	Sanada et al., 2012
S. enterica	SpvD	Xpo2 (nuclear exportin)	NF-κB	Reduces nuclear transport of NF-κB	Cysteine protease	Grabe et al., 2016; Rolhion et al., 2016

[a]Extracellular pathogen.

IFN response in the host and allows for Mtb DNA to be transferred into the host cytosol (Stanley et al., 2007; Manzanillo et al., 2012). cGAS binds mycobacterial DNA and colocalizes with Mtb-containing phagosomes as well as components of the host autophagy machinery (e.g., microtubule-associated protein 1A/1B-light chain 3 [LC3] and Beclin1); interestingly, chemical inhibition of autophagy interferes with cGAS localization to Mtb-containing phagosomes (Wassermann et al., 2015; Watson et al., 2015). Consistent with the role of cGAS in targeting Mtb for autophagy and lysosomal degradation, cGAS- and STING-deficient bone marrow–derived macrophages show reduced colocalization of autophagy markers (e.g., LC3) with Mtb-containing phagosomes and have an increased bacterial load 5 d postinfection (Watson et al., 2015).

STING is also activated by CDNs that are produced by bacteria (Burdette et al., 2011; Sauer et al., 2011). Both *Listeria monocytogenes* and *Chlamydia trachomatis* activate STING directly by secreting cyclic-di-AMP to induce a cGAS-independent IFN response (Woodward et al., 2010; Barker et al., 2013; Webster et al., 2017; Fig. 1 C). STING, as well as a recently discovered host protein, reductase controlling NF-κB (RECON), can bind to cyclic di-AMP and activate NF-κB independently of each other (Abe and Barber, 2014; McFarland et al., 2017). Whereas STING is a positive regulator of NF-κB that is activated by cyclic di-AMP, RECON is a negative regulator of NF-κB that is inhibited by cyclic di-AMP (McFarland et al., 2017). Despite the role of STING in innate immunity, STING-deficient mice do not display an increase in bacterial load or susceptibility to *L. monocytogenes* (Sauer et al., 2011; Collins et al., 2015). However, the cyclic di-AMP secreted by *L. monocytogenes* negatively impacts the T cell–mediated adaptive immune response to subsequent infections (Archer et al., 2014). Together, these studies highlight how cytosolic surveillance systems can also be modulated by bacterial metabolites and, in some cases, such as with *L. monocytogenes*, can dampen the host immune response.

Sensing of effector activity. In addition to PAMP recognition by PRRs, there is evidence to suggest that innate immune signaling pathways can recognize the enzymatic activity of certain bacterial effectors (Table 1). This phenomenon, termed effector-triggered immunity, was first described for plant–microbe interactions, whereby plants recognize pathogen-secreted effector proteins that suppress the initial PAMP-triggered immune response and counteract them by sending out an amplified, hypersensitive cell death response at the site of infection (Jones and Dangl, 2006). A similar exaggerated immune response to effector activity has been observed in the host response to infection by *Legionella pneumophila*. *L. pneumophila* uses a Dot/Icm type IV secretion system (T4SS) to deliver more than 300 effectors into the host cytosol during infection. Macrophages infected with wild-type *L. pneumophila* exhibit an exaggerated proinflammatory cytokine response in comparison to macrophages that are infected with the isogenic *L. pneumophila ΔdotA* strain, which lacks a T4SS (Shin et al., 2008). Although the majority of cytokines are produced through canonical TLR and NLR signaling pathways, the enhanced expression of certain proinflammatory cytokine genes (e.g., *IL-23a*, *Gem*, and *Csf2*) does not occur in macrophages that are infected with an *L. pneumophila* strain that lacks a set of five effectors (*Δ5*) that inhibit protein translation in the host (Fontana et al., 2011). This set of effectors includes three *Legionella* glucosyltransferase (Lgt) enzymes, which glucosylate and inactivate eukaryotic elongation factor 1A (eEF1A) in the host to inhibit translation (Belyi et al., 2006, 2008). Complementing the *Δ5* strain with wild-type Lgt effectors, but not with catalytically dead versions of these Lgt effectors, restores the enhanced cytokine expression in macrophages, suggesting that the induced inflammatory response depends on the glucosyltransferase activity of these Lgt effectors (Fontana et al., 2011, 2012). Likewise, enhanced cytokine expression is also restored in the *Δ5* strain with chemical inhibitors of protein translation (Fontana et al., 2011). Inhibiting protein synthesis results in prolonged activation of NF-κB by preventing the resynthesis of IκB (Fontana et al., 2011). The production of a subset of cytokines despite a global block in protein translation can be explained by the superinduction of specific cytokine transcripts when translation is inhibited; however, the details of the specific immune response pathways impacted by blocking translation remain to be explored (Barry et al., 2017). Interestingly, an enhanced immune response to *Clostridium difficile* is also dependent on the glucosyltransferase activity of the *C. difficile* effector, toxin A (TcdA); however, the effect of TcdA on protein translation is unknown (Cowardin et al., 2016). Similarly, inhibition of protein translation by the AB exotoxin (ToxA) of the extracellular pathogen *Pseudomonas aeruginosa* triggers the induction of a subset of immune responses in *Caenorhabditis elegans* (Dunbar et al., 2012; McEwan et al., 2012). Perturbing cellular homeostasis with specific effectors distinguishes pathogenic bacteria from commensal, nonpathogenic bacteria; therefore the ability to recognize the activity of pathogenic virulence factors serves an important role in host defense.

Bystander activation. Infected host cells can also signal to neighboring, uninfected cells to mount a proinflammatory cytokine response to assist in clearing infections by intracellular bacterial pathogens. This phenomenon, termed "bystander activation," has become increasingly important in understanding how multicellular organisms fight against microbial pathogens in the face of effector-mediated innate immune evasion (Holmgren et al., 2017). Uninfected bystander cells can respond to several signals that are emitted from infected cells, including reactive oxygen species (ROS), small molecules, PAMPs, and proinflammatory cytokines. For example, ROS intermediates produced by cells infected with *L. monocytogenes* can activate bystander cells to make proinflammatory CXCL2 and CXCL5 chemokines (Dolowschiak et al., 2010). *Mycobacterium*-infected macrophages are unable to produce the p40 subunit of IL-12 (IL-12p40) but release exosomes containing PAMPs to induce the production of IL-12p40 in bystander cells (Bhatnagar et al., 2007; Walters et al., 2013). As previously mentioned, effectors of *L. pneumophila* such as the Lgts target the host protein translation machinery, resulting in a major blockade in proinflammatory cytokine expression in infected host cells (Shin et al., 2008; Fontana et al., 2011, 2012; Table 1). However, by increasing the transcription of specific cytokines, the cells that are infected with *L. pneumophila* are able to overcome the global block in translation and produce a limited number of cytokines (e.g., IL-1α and IL-1β; Barry et al., 2017). These specific cytokines activate bystander cells to produce several of the cytokines that the infected cell cannot (i.e., IL-6 and IL-12; Asrat et al., 2014b; Copenhaver et al., 2015). Bystander cells can also be activated by small molecules (e.g., cGAMP or Ca^{2+}) that signal through gap junctions or by secreted macromolecules (e.g., inflammasomes or bacterial outer membrane vesicles; Holmgren et al., 2017). For example, the

cGAMP produced by cGAS activation appears to function in bystander activation. Although monocultures of cGAS-deficient or STING-deficient macrophages each have a reduced IFN response to infection with wild-type *M. tuberculosis*, infecting mixed cultures of these mutant cell lines partially restores IFN production (Wassermann et al., 2015). Furthermore, the partial restoration of the IFN response in mixed cultures is blocked by chemically inhibiting gap junction formation (Wassermann et al., 2015). In some cases, the signaling molecule for bystander activation is still unknown. For example, *Shigella flexneri* dampens the expression of IL-8 in infected host cells; however, MAPK-mediated IL-8 production is activated in neighboring, uninfected cells (Kasper et al., 2010). MAPKs (i.e., JNK, ERK, and p38) are activated in bystander cells in response to an unknown NOD1 activation signal that is transmitted through gap junctions, and IL-8 is produced by bystander cells in response to infections from several different bacterial pathogens (e.g., *S. flexneri*, *L. monocytogenes*, and *Salmonella typhimurium*; Kasper et al., 2010). Overall, bystander activation represents an important counterattack to effector-mediated inhibition of proinflammatory cytokine expression.

Bacterial inhibition of innate immunity

Inhibition of MAPK- and NF-κB–mediated proinflammatory responses that are activated downstream of TLRs and NLRs is a crucial survival strategy for bacterial pathogens (Fig. 1, A and B). As such, bacterial pathogens secrete effectors that either mimic host enzymes or use completely novel enzymatic activity to block innate immunity signaling (Table 1). The extracellular pathogen *Yersinia pestis* secretes a multifunctional enzyme, YopJ, that has serine/threonine acetyltransferase activity that inhibits several players of the MAPK and NF-κB signal transduction pathways (Orth et al., 1999; Mukherjee et al., 2006, 2008; Paquette et al., 2012; Fig. 1, A and B). Similarly, *S. typhimurium* secretes an acetyltransferase effector, AvrA, that is homologous to YopJ, which also targets MAPK signaling; however, rather than dampening proinflammatory cytokine expression, AvrA inhibits apoptosis to prolong host survival (Jones et al., 2008; Wu et al., 2012; Fig. 1 A). Both *S. flexneri* and *S. typhimurium* secrete effectors that block the MAPK-mediated proinflammatory cytokine response by dephosphorylating p38 and ERK MAPKs in the nucleus (Arbibe et al., 2007; Li et al., 2007; Zhu et al., 2007; Haneda et al., 2012; Fig. 1 A). Finally, *L. monocytogenes* effector internalin C (InlC) directly interacts with IKK to reduce the phosphorylation and subsequent degradation of IκB, thereby suppressing the activation of NF-κB (Gouin et al., 2010; Fig. 1 B). Infection with *L. monocytogenes inlC* mutants results in an increased proinflammatory response in macrophages as well as in mice (Gouin et al., 2010).

Bacterial pathogens modulate the ubiquitination of both positive and negative regulators of NF-κB to suppress the activation of NF-κB and the transcription of NF-κB–responsive genes. *S. flexneri* secretes multiple effectors (i.e., IpaH9.8, IpaH1.4, IpaH2.5, OspI, and OspG) that target the ubiquitination machinery of the host and block NF-κB activation (Fig. 1 B). For example, *S. flexneri* secretes an E3 ligase effector, IpaH9.8, that mimics host E3 ubiquitin ligases to target IKK, a critical activator protein complex of NF-κB, for proteasomal degradation (Ashida et al., 2010). *S. flexneri* secretes two additional E3 ligase effectors, IpaH1.4 and IpaH2.5, that target an essential subunit of the linear ubiquitin chain assembly complex (LUB AC) for proteasomal degradation and suppress the activation of NF-κB (de Jong et al., 2016). LUBAC is a multimeric, host E3 ubiquitin ligase that normally activates IKK with methionine 1–linked linear ubiquitin chains (Walczak et al., 2012). Another *S. flexneri* effector, OspI, is a glutamine deamidase that inactivates the E2 ubiquitin-conjugating enzyme Ubc13 and prevents the activation of the upstream regulator of NF-κB, TRAF6

Table 2. **Glossary of effectors: Histone modifications by bacterial virulence factors**

Bacteria	Effector	Host target	Target pathways	Outcome	Mode of action	Reference
L. monocytogenes	LLO	Unknown	Unknown	Dephosphorylation of H3S10; deacetylation of H4	Cholesterol-dependent cytolysin pore-forming toxin	Hamon et al., 2007
L. monocytogenes	InlB	SIRT2	PI3K/AKT	Deacetylation of H3K18	Binds to the cell surface receptor c-Met	Eskandarian et al., 2013
S. flexneri	OspF	ERK and p38 (MAPKs)	MAPK	Dephosphorylation of H3S10	Phosphothreonine lyase	Arbibe et al., 2007; Li et al., 2007; Zhu et al., 2007
S. typhimurium	SpvC	ERK (MAPKs)	MAPK	Dephosphorylation of MAPKs	Phosphothreonine lyase	Li et al., 2007; Zhu et al., 2007; Haneda et al., 2012
C. trachomatis	NUE	Histones H2B, H3, and H4	Direct PTM[a]	Transcriptional repression	Methyltransferase	Pennini et al., 2010
L. pneumophila Paris	RomA	Histone H3	Direct PTM	Methylation of H3K14; transcriptional repression	Methyltransferase	Rolando et al., 2013
L. pneumophila Philadelphia LP02	LegAS4	Histone H3	Direct PTM	Methylation of H3K4; transcriptional activation of ribosomal RNA genes	Methyltransferase	Li et al., 2013
B. anthracis[b]	BaSET	Histone H1	Direct PTM; NF-κB	Trimethylation of histone H1; transcriptional repression of NF-κB target genes	Methyltransferase	Mujtaba et al., 2013
M. tuberculosis	Rv1988	Histone H3	Direct PTM	Dimethylation of histone H3R42; transcriptional repression of genes involved in ROS production	Methyltransferase	Yaseen et al., 2015

[a]PTM, posttranslational modification.
[b]Extracellular pathogen.

(Sanada et al., 2012; Fig. 1 B). Finally, OspG interferes with NF-κB activation by preventing the degradation of IκB (Kim et al., 2005; Zhou et al., 2013; Fig. 1 B). OspG binds to both ubiquitin and the E2-ubiquitin conjugating enzymes, which allosterically activates the OspG kinase domain and is required to block IκB degradation (Kim et al., 2005; Zhou et al., 2013; Pruneda et al., 2014). *S. flexneri* OspG mutants induce a stronger inflammatory response than wild-type *S. flexneri* because they are unable to prevent the dissociation of NF-κB from IκB (Kim et al., 2005; Zhou et al., 2013).

The translocation of NF-κB to the nucleus depends on the proper recycling of nuclear proteins, a process that is facilitated by importins and exportins, which shuttle proteins in and out of the nucleus, respectively. An imbalance of importin/exportin shuttling disrupts NF-κB transport into the nucleus (Rolhion et al., 2016). The *S. typhimurium*–secreted effector SpvD binds the nuclear exportin, Xpo2, which results in the accumulation of importin-α in the nucleus and reduces transport of NF-κB to the nucleus (Rolhion et al., 2016; Fig. 1 B). The crystal structure and mutational analysis of SpvD suggest that SpvD is a cysteine protease; however, exactly how this protease function negatively regulates NF-κB signal transduction is still unknown (Grabe et al., 2016). Overall, many intracellular bacterial pathogens secrete effectors that target MAPK and NF-κB signaling pathways to counteract the deleterious output of these host defense pathways and prevent downstream proinflammatory cytokine expression.

Histone modifications by bacterial virulence factors

MAPK-mediated innate immune signaling pathways also introduce or remove posttranslational modifications onto histones to change the chromatin structure and facilitate the transcription of proinflammatory cytokines. In eukaryotes, DNA is packaged into nucleosomes, which consist of an octamer of core histones (i.e., H2A, H2B, H3) that are linked together by histone H1 into higher-order assemblies (Luger et al., 1997; Fig. 1 D). Posttranslational modifications to histones (e.g., methylation, phosphorylation, acetylation, and ubiquitination) greatly impart an additional level of transcriptional regulation by dictating the accessibility of transcriptional activators and repressors to a given promoter. For example, MAPK-mediated phosphorylation of serine 10 on histone H3 (H3S10) increases the accessibility of NF-κB to the promoters of certain cytokines, including IL-8; in fact, global increase in phosphorylation of H3S10 is observed when cells are exposed to even LPS alone (Saccani et al., 2002). However, several intracellular bacterial pathogens secrete effectors that can counteract host histone modifications to dampen the expression of proinflammatory cytokines (Table 2).

Histone modifications via innate immune signaling pathways. *L. monocytogenes* induces a proinflammatory cytokine response upon infection via canonical MAPK-mediated histone modifications (i.e., phosphorylation of H3S10) at the promoter regions of NF-κB regulated genes (i.e., IL-8; Schmeck et al., 2005; Opitz et al., 2006; Hamon et al., 2007). However, *L. monocytogenes* is able to quickly dampen the host immune response by dephosphorylating H3S10 (Hamon et al., 2007). In addition, *L. monocytogenes* also globally deacetylates H3 and H4 (Hamon et al., 2007; Fig. 1 D). The dephosphorylation of H3S10 and deacetylation of H4 are dependent on the *L. monocytogenes*–secreted virulence factor listeriolysin O (LLO; Hamon et al., 2007; Fig. 1 D). In the presence of LLO, specific proinflammatory genes (i.e., *cxcl2* and *dusp4*) are transcriptionally down-regulated and show reduced levels of both phosphorylated H3S10 and acetylated H4 (Hamon et al., 2007). Therefore, it appears that LLO-induced histone modifications impart a specific transcriptional response in the host. Interestingly, H3S10 dephosphorylation by *L. monocytogenes* does not depend on its ability to enter the cell or to damage the cell membrane; however, it does depend on the membrane-binding ability of LLO, suggesting that LLO possibly modulates host signal transduction pathways to induce histone modifications (Hamon et al., 2007). LLO is a member of a family of cholesterol-dependent cytolysin pore-forming toxins that is shared by other bacterial pathogens. Remarkably, purified cholesterol-dependent cytolysin toxins from two different extracellular pathogens, *Clostridium perfringens* and *Streptococcus pneumonia,* also reduced the levels of global H3S10 to a similar extent as LLO, which suggests that other bacterial pathogens possess the ability to epigenetically modulate host gene expression by altering histone modifications in a similar way (Hamon et al., 2007). Another *L. monocytogenes* effector, internalin B (InlB), also induces deacetylation of histone 3 on lysine 18 (H3K18) by activating a host histone deacetylase, sirtuin 2 (SIRT2; Eskandarian et al., 2013; Fig. 1 D). Deacetylation and occupancy by SIRT2 at transcriptional start sites of many genes involved in immune response regulation correlated with the transcriptional repression during *L. monocytogenes* infection (Eskandarian et al., 2013). Likewise, the loss or inhibition of SIRT2 greatly attenuates infection by *L. monocytogenes* (Eskandarian et al., 2013).

S. flexneri also inhibits MAPK signaling pathways to alter the epigenetic control of cytokine expression. *S. flexneri* secretes a unique phosphothreonine lyase effector, OspF, which dephosphorylates MAPKs (i.e., p38 and ERK) in the nucleus (Fig. 1 A). MAPK inactivation by OspF, in turn, reduces phosphorylation of H3S10 at the promoters of NF-κB regulated genes (e.g., IL-8) and attenuates the binding of NF-κB to these promoters (Arbibe et al., 2007; Fig. 1 D). Unlike traditional phosphatases, OspF irreversibly dephosphorylates host MAPKs using a phosphothreonine lyase mechanism that has not yet been described for any eukaryotic host enzymes; therefore, it represents an irreversible catalytic mechanism used by a bacterial pathogen to target host MAPKs and inflict effector-mediated inhibition of host immunity (Li et al., 2007; Zhu et al., 2007). *S. typhimurium* also secretes a similar phosphothreonine lyase effector, SpvC, which dephosphorylates a MAPK (i.e., ERK) to reduce inflammation and promote bacterial replication in vivo (Li et al., 2007; Zhu et al., 2007; Haneda et al., 2012; Fig. 1 A).

Direct modification of histones by secreted effectors. Recently, several bacterial methyltransferases have been identified that can localize to the nucleus and methylate mammalian host histones (Table 2). These bacterial methyltransferases share a conserved SET domain, which catalyze the attachment of a methyl group onto lysine residues of histones using a *S*-adenosyl-L-methionine (SAM) methyl donor. The first bacterial histone lysine methyltransferase (HKMT) effector was discovered in *C. trachomatis* and termed nuclear effector (NUE; Pennini et al., 2010). NUE is secreted by the *C. trachomatis* type III secretion system (T3SS) and localizes to the host cell nucleus. Interestingly, NUE exhibits automethylation activity, which improves its ability to methylate H2B, H3, and H4 in vitro (Pennini et al., 2010; Fig. 1 D). *L. pneumophila* Paris and *L. pneumophila* Philadelphia Lp02 strains possess

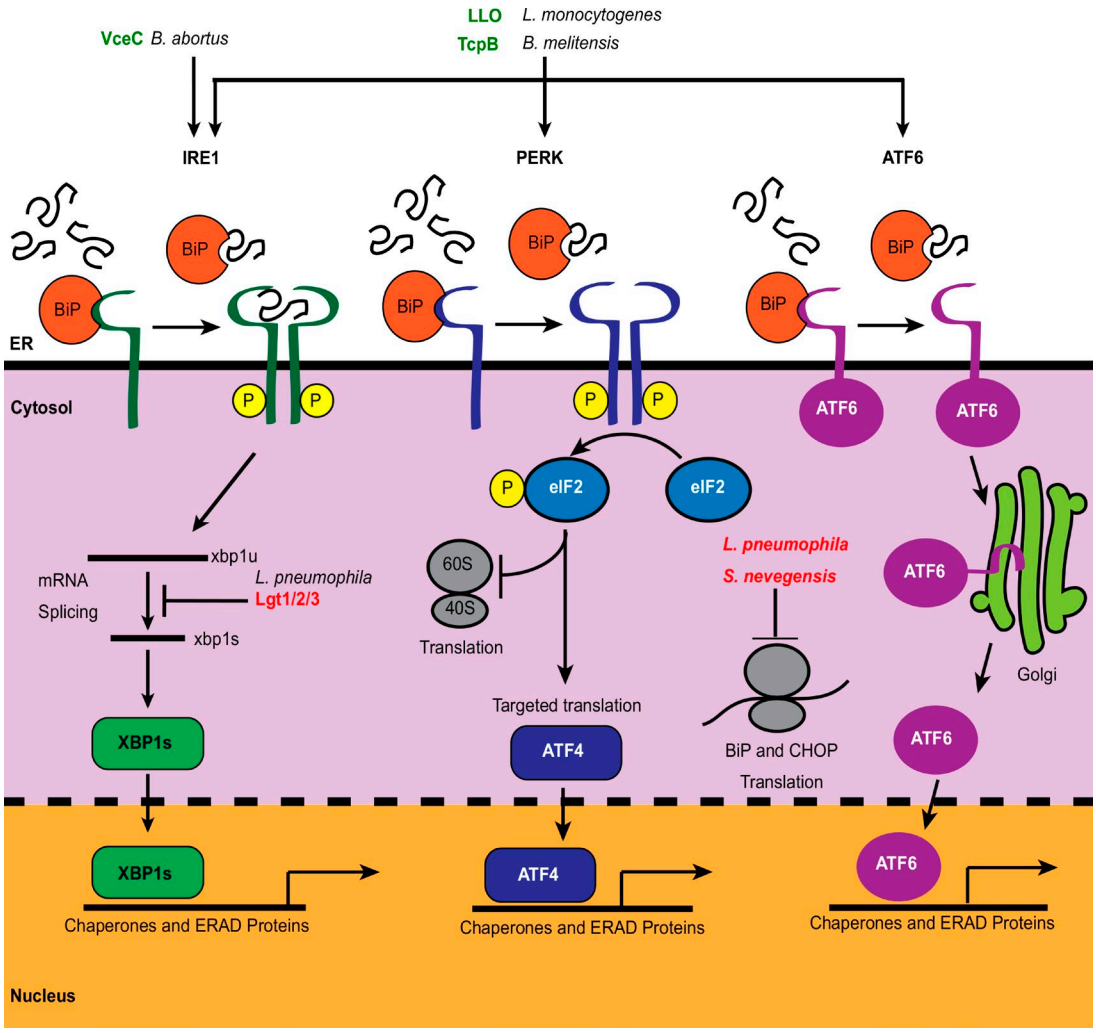

Figure 2. **Modulation of the UPR by bacterial pathogens.** The UPR is mediated by three major sensors in the ER: IRE1, PERK, and ATF6. In the presence of unfolded proteins, the ER resident chaperone, BiP, dissociates from these UPR sensors, which contributes to their activation and downstream cellular responses, which include expression of protein chaperones and ERAD. Bacterial pathogens both activate (green) and inhibit (red) all three branches of the UPR.

homologous HKMT effectors, RomA and LegAS4, respectively, which exert a strain-dependent phenotype on the host (Li et al., 2013; Rolando et al., 2013). Although both effectors methylate H3 to alter host transcription, they target distinct residues. RomA localizes to the nucleus and methylates histone 3 lysine 14 (H3K14), which results in global transcriptional repression (Rolando et al., 2013; Fig. 1 D). H3K14 histone methylation is a novel epigenetic mark that appears to compete with H3K14 histone acetylation of the mammalian host (Rolando et al., 2013). In contrast, LegAS4 localizes to the nucleolus and methylates histone 3 lysine 4 (H3K4), which results in increased transcription of ribosomal RNA genes (rDNA; Li et al., 2013; Fig. 1 D). It is worth noting that an increase in H3K14 methylation is also observed by immunofluorescence upon infection with wild-type *L. pneumophila* Philadelphia Lp02; however, whether H3K14 methylation depends on LegAS4 remains to be determined (Rolando et al., 2013). Many bacterial pathogens contain HKMT homologues in their effector repertoire, suggesting that histone methylation might be a widespread strategy to take advantage of host transcription (Li et al., 2013). For

Table 3. **Glossary of effectors: Bacterial modulation of the UPR**

Bacteria	Effector	Host target	Target pathways	Outcome	Mode of action	Reference
B. melitensis	TcpB	Unknown	ATF6, PERK, IRE1	Activation of the UPR	Unknown	Smith et al., 2013
B. abortus	VceC	BiP	IRE1	Activation of the UPR	Unknown	de Jong et al., 2013; Keestra-Gounder et al., 2016
L. monocytogenes	LLO	Unknown	ATF6, PERK, IRE1	Activation of the UPR	Unknown	Pillich et al., 2012
L. pneumophila	Lgt1, Lgt2, Lgt3	Unknown	IRE1	Inhibition of XBP1u mRNA splicing	Glucosyltransferase	Hempstead and Isberg, 2015; Treacy-Abarca and Mukherjee, 2015

example, a SET domain containing an effector protein was recently identified in *Bacillus anthracis* (BaSET), an extracellular bacterial pathogen that specifically trimethylates histone H1, but not the core histones (H2A, H2B, H3, and H4) in vitro (Mujtaba et al., 2013; Fig. 1 D). Deleting *BaSET* renders *B. anthracis* avirulent, and transient overexpression of *BaSET* in mammalian cells was capable of repressing the expression of NF-κB and NF-κB target genes (Mujtaba et al., 2013). Finally, *M. tuberculosis* secretes an effector methyltransferase, Rv1988, which dimethylates histone H3 on arginine 42 (H3R42me2) to repress the transcription of genes involved in producing ROS (Yaseen et al., 2015; Fig. 1 D). Because ROS production is a crucial host defense against bacterial pathogens, it is not surprising that deleting *rv1988* from *M. tuberculosis* attenuates bacterial survival in host macrophages (Yaseen et al., 2015). Unlike most known regulatory histone modifications, this modification does not occur on the N termini of histones, but rather on a histone residue that is critical for DNA entry/exit from the nucleosome. Thus, Rv1988 is a novel virulence factor that imparts a noncanonical histone modification to modulate host immunity. Overall, by altering posttranslational modifications on histones via either MAPK signaling pathways or molecular mimicry, intracellular bacterial pathogens can inhibit the proinflammatory response of the host and manipulate host gene expression to their advantage.

Intracellular bacteria modulate the UPR

The UPR. Precise quality control of protein synthesis ascertains that only correctly folded proteins exit the ER (Schwarz and Blower, 2016). If cellular homeostasis is disturbed by physiological stress (e.g., DNA damage, chemical stimuli, or pathogen infection), misfolded and unfolded proteins accumulate in the lumen of the ER and cause ER stress. As a response, an evolutionarily conserved signaling network, the UPR pathway, is activated to alleviate this imbalance and restore ER homeostasis. The UPR pathway down-regulates overall protein translation, induces ER-associated protein degradation (ERAD) of aberrantly folded proteins, and increases the synthesis of chaperones responsible for protein folding (Walter and Ron, 2011). The UPR is controlled by a set of three transmembrane ER-resident proteins: inositol-requiring protein 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6; Fig. 2). Under unstressed conditions, the luminal domains of these three sensors are stably bound to the ER chaperone immunoglobulin binding protein (BiP), which dissociates from its partners during ER stress, contributing to their activation (Gardner et al., 2013).

IRE1 is an ER transmembrane kinase that, upon sensing ER stress, oligomerizes and autophosphorylates to activate its RNase domain. The RNase domain is located on the cytosolic surface and targets X-box–binding protein 1 mRNA (XBP1u), resulting in spliced XBP1 mRNA (XBP1s). XBP1s mRNA encodes a transcription factor that is responsible for up-regulating UPR target genes that foster ERAD and enhance overall ER protein folding capacity (Cox and Walter, 1996; Yoshida et al., 2001; Korennykh et al., 2009). Similar to IRE1, PERK is also an ER transmembrane kinase that oligomerizes and autophosphorylates upon activation. Activated PERK phosphorylates the α-subunit of the eukaryotic initiation factor 2 (eIF2), which leads to a reduction of the ER workload by attenuating global mRNA translation. During this time, some mRNAs are preferentially translated, e.g., ATF4, which is responsible for the induction of several UPR target genes such as C/EBP homologous protein (CHOP; Harding et al., 1999, 2000). The third regulator, ATF6, translocates to the Golgi and is proteolytically cleaved upon activation, resulting in an active b-ZIP transcription factor that is responsible for the induction of several UPR target genes (Haze et al., 1999; Adachi et al., 2008). If the ER stress remains unresolved, the UPR pathway will finally lead to the induction of apoptosis (Iurlaro and Muñoz-Pinedo, 2016).

UPR activation by intracellular bacteria. Given the central roles of the UPR in managing ER homeostasis and responding to cellular stress, it is not surprising that several pathogens have developed strategies to actively manipulate the UPR to their advantage (Table 3). Under certain instances, inducing the UPR appears to actually promote bacterial replication; therefore, it is still unclear whether effector-induced UPR is a strategy for intracellular pathogens to increase ER folding capacity for their benefit, or whether this in a consequence of the robust defense system of the host. For example, secretion of LLO by *L. monocytogenes* activates all three branches of the UPR, and chemically inducing ER stress during *L. monocytogenes* infection attenuates bacterial survival (Pillich et al., 2012). On the other hand, both *Brucella melitensis* and *Brucella abortus* activate branches of the UPR, and pharmacologically blocking the UPR during infection significantly impairs intracellular replication of *Brucella* (Smith et al., 2013; Keestra-Gounder et al., 2016). *B. melitensis* infection turns on all three branches of the UPR (Smith et al., 2013; Fig. 2). Activation of the IRE1 branch is most likely mediated by TLRs, because XBP1u mRNA splicing is dependent on the TLR adapter protein myeloid differentiation primary response gene 88 (MyD88) and still occurs when cells are treated with heat-killed *B. melitensis* (Smith et al., 2013). However, the induction of UPR target genes BiP, CHOP, and ER DnaJ-like 4 (ERdj4) does not depend on MyD88, but instead, requires the *B. melitensis* protein TcpB (Fig. 2). Even incubating macrophages with purified TcpB protein induces UPR gene expression and restructuring of the ER (Smith et al., 2013).

On the other hand, *B. abortus* specifically activates the IRE1 branch of the UPR, but not the ATF6 or PERK branches. Activation of the IRE1 pathway by *B. abortus* up-regulates the machinery needed for coat protein complex II (COPII) secretory vesicle formation at ER exit sites, which *B. abortus* uses to form its ER-derived replicative vacuole (Taguchi et al., 2015). Formation of large ER vacuoles under UPR-inducing conditions requires the YPT-interacting protein 1A (Yip1A), a positive regulator of IRE1. Knocking down either Yip1A or IRE1 during infection inhibits the intracellular replication of *B. abortus*, suggesting that *B. abortus* exploits the IRE1 branch of the UPR to build a replicative intracellular niche (Taguchi et al., 2015).

IRE1 is activated by the *B. abortus*–secreted effector VceC, which binds to BiP inside the ER lumen (Fig. 2). In addition, ectopic expression of VceC induces structural reorganization of the ER (de Jong et al., 2013). UPR induction is concomitant with IRE1-dependent proinflammatory cytokine expression (de Jong et al., 2013). It was recently shown that VceC induces this proinflammatory cytokine expression through a noncanonical pathway that is triggered by IRE1 activation of Nod1/Nod2 innate immune signaling (Keestra-Gounder et al., 2016). The expression of IL-6 is drastically reduced in cells infected with *B. abortus vceC* mutants compared with cells infected with wild-type *B. abortus* or cells in which Nod2 signaling is induced with muramyl dipeptide (Keestra-Gounder et al., 2016).

In vivo studies show that necrosis is reduced and the survival of pups increases when mice are infected with the *B. abortus vceC* mutant (Keestra-Gounder et al., 2016). Remarkably, blocking the UPR with the general UPR inhibitor, tauroursodeoxycholic acid, during infection with wild-type *B. abortus* also increases survival and reduces necrosis, suggesting that pharmacologically inhibiting the UPR could be a viable option for treating *B. abortus* infections (Keestra-Gounder et al., 2016).

A noncanonical role of the UPR in the innate immune response to pathogens via cross talk with TLR and NLR signaling pathways is beginning to emerge (Celli and Tsolis, 2015). For example, activated TLR4 and TLR2 are responsible for IRE1 phosphorylation and subsequent XBP1u mRNA splicing (Iwakoshi et al., 2007). Remarkably, stimulating TLR4 and TLR2 with receptor agonist activates the IRE1 pathway independently of chemically induced ER stress; surprisingly, however, TLR-dependent activation of IRE1 does not induce the canonical downstream ER stress response (Martinon et al., 2010). Rather than inducing the transcription of canonical UPR target genes (i.e., BiP, CHOP, and ERdj4), TLR-dependent activation of IRE1 leads to elevated production of proinflammatory cytokines (e.g., IL-6; Martinon et al., 2010).

There is also evidence of cross talk between activated TLRs and the PERK arm of the UPR pathway. Stimulation of TLR4 by LPS increases phosphorylation of both PERK and its downstream target eIF2 (Woo et al., 2009). However, despite activation of the PERK pathway by LPS, TLR signaling blocks the expression of the downstream UPR target gene CHOP (Woo et al., 2009). TLR signaling inhibits the PERK pathway by post-translationally modifying eIF2B, the activator of eIF2, which allows eIF2B to avoid competitive inhibition by phosphorylated eIF2 (Woo et al., 2012). Thus, although stimulation of TLRs activates the sensor kinases of the UPR (i.e., IRE1 and PERK), it appears that in most cases TLR activation inhibits expression of canonical UPR target genes and, instead, synergizes with noncanonical UPR pathways to mount a robust proinflammatory response against bacterial pathogens. The molecular cross talk between the UPR and innate immune signaling pathways is only beginning to emerge and represents yet another important intersection of host defense against bacterial pathogens.

UPR inhibition by intracellular bacteria. Because of the especially important role of the UPR in sensing invading pathogens and in the defense response to bacterial infection, it is not surprising that some pathogens have managed to figure out how to usurp UPR activation. *L. pneumophila* is one such pathogen; induction of the UPR with chemical inducers of ER stress is strongly inhibited in cells infected with *L. pneumophila* (Hempstead and Isberg, 2015; Treacy-Abarca and Mukherjee, 2015). Inhibition of the UPR is effector mediated, as the *ΔdotA* strain, which lacks a functional T4SS, is unable to block the UPR (Hempstead and Isberg, 2015; Treacy-Abarca and Mukherjee, 2015). Although the exact mechanism of inhibition is not fully understood, *L. pneumophila* secretes three glucosyltransferase effector proteins that block the IRE1 branch of the UPR by inhibiting the splicing of XBP1u mRNA (Hempstead and Isberg, 2015; Treacy-Abarca and Mukherjee, 2015; Fig. 2 and Table 2). Moreover, additional unknown *L. pneumophila* effectors inhibit the translation of BiP and CHOP (Treacy-Abarca and Mukherjee, 2015; Fig. 2). Notably, ATF6 processing, as well as the transcription of BiP and CHOP, increases upon *L. pneumophila* infection. It is unclear whether the translation of all of the downstream targets of ATF6 is also suppressed, or whether *L. pneumophila* only selectively blocks the translation of BiP and CHOP. *L. pneumophila* secretes five effectors that are known to inhibit global protein translation; however, these effectors do not appear to be responsible for blocking BiP translation, because the *Δ5 L. pneumophila* mutant that lacks these five effectors still inhibits the translation of BiP (Treacy-Abarca and Mukherjee, 2015). The translation of several UPR targets, including BiP, is controlled by noncanonical translation initiation factors that target upstream ORFs during the UPR (Starck et al., 2016). Therefore one possibility is that *L. pneumophila* effectors target these noncanonical modes of translation; however, this possibility remains to be explored.

The UPR was also shown to be inhibited in cells infected with *Simkania negevensis*, an intracellular, Gram-negative bacterial pathogen of the order *Chlamydiales* (Mehlitz et al., 2014). *Simkania*-containing vacuoles form a continuous network that interacts extensively with the host ER. Like *L. pneumophila*, *S. negevensis* triggers BiP transcription early in infection; however, BiP translation is later inhibited (Mehlitz et al., 2014; Fig. 2). *S. negevensis* also blocks the translocation of preexisting CHOP protein to the nucleus (Mehlitz et al., 2014). Furthermore, phosphorylated eIF2 levels are reduced during infection, suggesting that *S. negevensis* may also inhibit the PERK-mediated branch of the UPR (Mehlitz et al., 2014). The ability of *S. negevensis* to inhibit the host UPR was essential for forming a replicative vacuole (Mehlitz et al., 2014). Inactivation of PERK seems to benefit other intracellular pathogens as well. The inability to activate PERK because of defective eIF2 phosphorylation results in a higher intracellular bacterial load of *L. monocytogenes* and *C. trachomatis* (Shrestha et al., 2012). The differences by which pathogens manipulate the UPR most likely reflect their specific requirements for establishing an intracellular niche; some pathogens might exploit the UPR to take advantage of its homeostatic function (i.e., increased protein folding capacity and lipid biosynthesis), whereas other pathogens may opt to block certain branches of the UPR altogether to avoid its role in host defense, such as apoptosis or innate immunity (Celli and Tsolis, 2015). Both the degree and duration of UPR activation dictates these diverse outcomes (Walter and Ron, 2011). Therefore, understanding the mechanisms by which pathogens spatially and temporally manipulate the UPR could shed light on how the distinct outcomes of the UPR are regulated by the cell in the context of infection.

Bacterial manipulation of the autophagy pathway

Another crucial homeostatic process that is often targeted by invading pathogens to promote their survival and growth is autophagy. Autophagy is a catabolic process responsible for the lysosomal degradation of different cytoplasmic components (e.g., dysfunctional organelles and proteins) to recycle and provide new building blocks for the cell. Moreover, autophagy also has an important role in restricting intracellular growth of many bacteria by a selective antipathogenic form of autophagy, called xenophagy. Also, in vivo studies showed that autophagy protects against the dissemination of intestinal bacteria (Benjamin et al., 2013). This host defense mechanism uses the autophagy machinery to specifically target invading pathogens for lysosomal degradation (Huang and Brumell, 2014).

In principle, xenophagy follows the basic steps of the autophagy pathway (Fig. 3), which can be induced by a variety

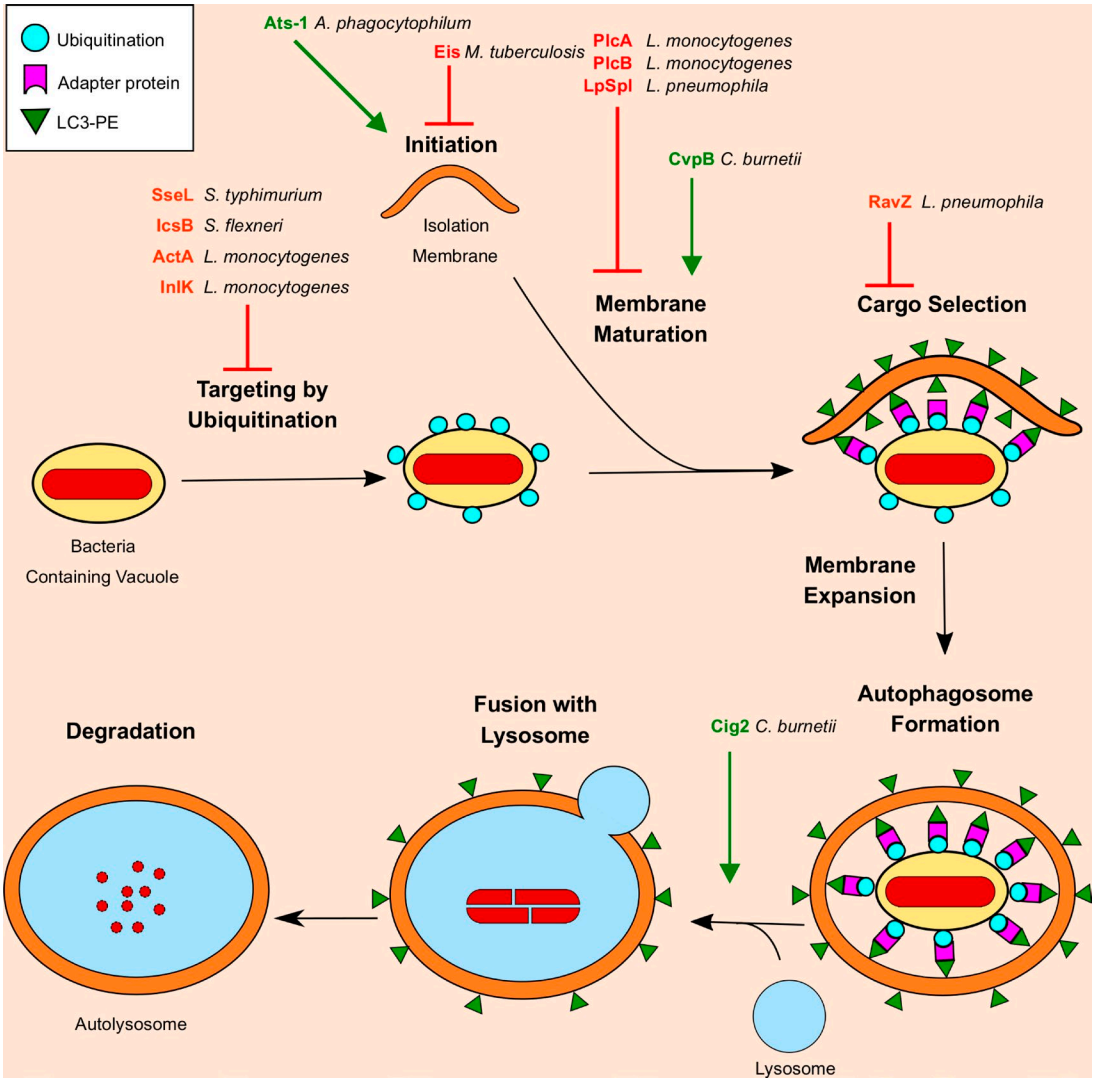

Figure 3. **Modulation of the autophagy pathway by bacterial pathogens.** After invasion of the host cell, vacuoles containing intracellular bacteria are targeted for the autophagy machinery by ubiquitination. Adapter proteins specifically direct ubiquitinated bacteria containing vacuoles to LC3–PE conjugates on mature isolation membranes. The membrane expands and forms a double-membrane compartment called an autophagosome, which eventually fuses with lysosomes and leads to the degradation of its bacterial cargo. Several bacteria have evolved different effector proteins that inhibit (red) this selective autophagic immune mechanism. However, some bacterial pathogens secrete effectors that exploit this pathway (green) and make use of the generated nutrients and membranes to promote their intracellular growth.

of signals, such as the detection of invading bacteria by PRRs (Deretic et al., 2013; Cadwell, 2016). After induction, several autophagy-related (Atg) proteins are recruited to the isolation membrane that forms around cytoplasmic components. Expansion of the isolation membrane and formation of the double-membrane autophagosome are mediated by ubiquitin-like conjugation systems that facilitate the addition of phosphatidyl-ethanolamine (PE) to LC3 on the isolation membrane (Fig. 3). The LC3–PE conjugate has a critical role in the selection of the cargo. Once detected, intracellular bacteria are ubiquitinated by ubiquitin ligases, such as LRSAM1 and Parkin (Huett et al., 2012; Manzanillo et al., 2013). Ubiquitin-binding adapter proteins bearing an LC3-interacting region (LIR; e.g., p62, NBR1, NDP52, and optineurin) then direct these ubiquitin-tagged bacteria to the developing autophagosome (Johansen and Lamark, 2011). Additionally, vacuoles containing bacteria can be marked for lysosomal fusion by direct recruitment of LC3 without ubiquitination through a noncanonical autophagy process called LC3-associated phagocytosis (LAP; Birgisdottir et al., 2013). The final step of the autophagic pathway includes the fusion of the autophagosome with lysosomes to generate the autolysosome, where bacteria are degraded by hydrolytic enzymes (Kimmey and Stallings, 2016; Fig. 3). Enabling autophagic pathways restricts growth and proliferation of several intracellular bacterial pathogens. However, many intracellular bacteria have developed ways to manipulate xenophagy at different steps of the process to survive and replicate inside of the host cell (Table 4).

Evasion of xenophagy. Several studies have investigated how bacteria are able to inhibit xenophagy to promote their intracellular growth. Some bacteria interfere with the signaling cascade leading to the initiation of autophagy. One of the autophagy triggers is the production of ROS; likewise some bacteria have evolved ways to down-regulate ROS production (Rabadi et al., 2016). For example, the *M. tuberculosis N*-acetyltransferase effector Eis activates a JNK-specific phosphatase

that leads to the inactivation of JNK and subsequent blocking of ROS production (Kim et al., 2012; Fig. 3).

One of the most crucial steps in the autophagy pathway is the selection of bacterial cargo by ubiquitination, which targets the bacteria-containing vacuole to the developing autophagosome via interactions between ubiquitin, adapter molecules, and LC3-PE (Fig. 3). Several bacteria have evolved interesting strategies to interfere with this critical process to promote escape from xenophagy at this initial step. For example, *S. typhimurium* is able to degrade ubiquitinated protein aggregates that form around the *Salmonella*-containing vacuole (SCV), which would be normally recognizable by the autophagy machinery. *S. typhimurium* secretes the effector protein, SseL, that deubiquitinates ubiquitin aggregates and thereby decreases the recruitment of the SCV to the autophagosome by the ubiquitin–adapter protein–LC3 interaction (Mesquita et al., 2012; Fig. 3). Another common strategy of autophagy evasion is the modification of the bacterial surface to diminish ubiquitin tagging, as exemplified by *S. flexneri* or *L. monocytogenes* (Ogawa et al., 2005; Yoshikawa et al., 2009; Dortet et al., 2011). Recognition of the *S. flexneri* membrane protein VirG by host Atg5 induces xenophagy. However, *S. flexneri* is able to escape xenophagy by secreting the effector IcsB, which binds competitively to VirG and thereby shields the bacterium from getting marked by Atg5 (Ogawa et al., 2005; Fig. 3). In contrast, *L. monocytogenes* uses host proteins to camouflage its surface and escape ubiquitin tagging. *L. monocytogenes* secretes the virulence factor InlK, which helps to mask its cell surface by binding the mammalian cytoplasmic protein major vault protein (MVP) to reduce ubiquitination and avoid xenophagy (Dortet et al., 2011; Fig. 3). Another *L. monocytogenes* effector protein, ActA, mediates protection from xenophagy by recruiting the Arp2/3 complex and Ena/VASP proteins to the bacterial surface, thereby avoiding recognition, ubiquitination, and the recruitment of adapter proteins and LC3 (Yoshikawa et al., 2009; Fig. 3). Moreover, expression of ActA maintains the actin-based motility and ability for *L. monocytogenes* to disseminate within and between cells (Mostowy et al., 2011). Many pathogens that use actin-based motility (e.g., *S. flexneri*) recruit septins that assemble into septin cage-like structures to entrap actin-polymerizing bacteria. These assemblies are recognized by the adapter proteins p62 and NDP52 and subsequently targeted for autophagy (Mostowy et al., 2010, 2011). Mitochondria support septin-cage assembly; however, *Shigella*-induced mitochondria fragmentation leads to escape from these cages and avoidance of autophagy induction (Krokowski et al., 2016).

A second common mechanism used by bacteria to avoid autophagic recognition is to inhibit the formation of the LC3–PE conjugate on the autophagosome membrane. For example, *L. monocytogenes* secretes the phospholipases PlcA and PlcB that prevent the formation of phosphatidylinositol 3-phosphate (PI3P) and thereby block LC3 lipidation (Tattoli et al., 2013; Mitchell et al., 2015; Fig. 3). Invading bacteria also interfere with the metabolism of sphingolipids, a class of bioactive lipids that are required for LC3 lipidation and induction of autophagy (Young et al., 2013). For example, *L. pneumophila* disrupts host sphingolipid metabolism by secreting the sphingosine-1 phosphate lyase (LpSpl) that down-regulates host sphingolipid levels and causes a delay in the autophagic response (Rolando et al., 2016; Fig. 3).

However, all of the bacterial strategies to avoid xenophagic degradation described here are indirect effects on the

Table 4. **Glossary of effectors: Bacterial manipulation of the autophagy pathway**

Bacteria	Effector	Host target	Target pathways	Outcome	Mode of action	Reference
M. tuberculosis	Eis	JNK specific phosphatase (DUSP16/ MKP-7)	MAPK (i.e., JNK); ROS generation; autophagy	Inhibition of autophagy	*N*-acetyltransferase	Kim et al., 2012
S. typhimurium	SseL	Ubiquitin	Autophagy	Degradation of ubiquitinated protein aggregates on the SCV	DUB	Mesquita et al., 2012
S. flexneri	IcsB	None	Autophagy	Camouflaging of bacterial surface protein VirG	Unknown	Ogawa et al., 2005
L. monocytogenes	InlK	MVP	Autophagy	Camouflaging of bacterial surface proteins	Unknown	Dortet et al., 2011
L. monocytogenes	ActA	Arp2/3 complex; Ena/ VASP proteins	Autophagy	Recruitment of host proteins for camouflaging; prevention of septin cage formation	Unknown	Yoshikawa et al., 2009; Mostowy et al., 2011
L. monocytogenes	PlcA, PlcB	PI3P	Autophagy	Inhibition of LC3 lipidation	Phospholipases	Tattoli et al., 2013; Mitchell et al., 2015
L. pneumophila	LpSpl	Sphingolipid production	Autophagy	Inhibition of LC3 lipidation	Sphingosine-1 phosphate lyase	Rolando et al., 2016
L. pneumophila	RavZ	LC3	Autophagy	Cleavage of lipidated LC3	Cysteine protease	Choy et al., 2012; Horenkamp et al., 2015; Yang et al., 2017
Group A Streptococcus[a]	SpeB	p62, NDP52, NBR1	Autophagy	Degradation of adapter proteins required for autophagy induction	Cysteine protease	Barnett et al., 2013
A. phagocytophilum	Ats-1	BECN1	Autophagy	Induction of autophagosome formation	Unknown	Niu et al., 2012; Niu and Rikihisa, 2013
C. burnetii	Cig2	LC3	Autophagy	Enhanced fusion of autophagosomes with the CCV	Unknown	Newton et al., 2014
C. burnetii	CvpB	PI3P; phosphatidyl-inositol 5-kinase PIKfyve	Autophagy	Enhanced association of the autophagy machinery to CCVs; homotypic fusion of CCVs	Unknown	Martinez et al., 2016

[a]Extracellular pathogen.

components of the autophagy machinery. To date, there are only a few examples of bacterial effector proteins that have a direct biochemical effect on a major autophagy component. One example is the *L. pneumophila* cysteine protease RavZ, which gets secreted by a T4SS and localizes to the autophagosome. RavZ extracts LC3-PE from the membrane, irreversibly cleaves lipidated LC3, and thereby removes it from the autophagosomal membrane (Choy et al., 2012; Yang et al., 2017; Fig. 3). A unique combination of a PI3P binding and protease domain, as well as an amphipathic loop that anchors it in the autophagosomal membrane, allows RavZ to induce a global shutdown of xenophagy (Horenkamp et al., 2015). Moreover, some strains of the extracellular Group A *Streptococcus* (GAS) express SpeB, a streptococcal cysteine protease. SpeB degrades the adapter proteins p62, NDP52, and NBR1 both within the host cell cytosol and in vitro. By degrading autophagic adapter proteins, GAS is not targeted to the developing autophagosome and successfully escapes xenophagy (Barnett et al., 2013; Fig. 3).

Exploitation of the autophagy machinery. In contrast to bacteria that inhibit autophagy to secure their survival, other intracellular bacteria induce autophagy to promote infection. Some bacteria appear to have evolved strategies to hijack the autophagosomes to gain access to recycled nutrients that are normally used by the host cell. Indeed, the absence of autophagy induction can be directly correlated with an impaired life cycle and reduced growth for several bacteria (Escoll et al., 2016).

The intracellular bacterium *Anaplasma phagocytophilum* replicates in host-derived double-membrane bound vacuoles. These vacuoles are similar to the autophagosomes that harbor LC3 and beclin-1 (BECN1), a critical protein in the induction of autophagy and membrane nucleation. *A. phagocytophilum* secretes the effector protein Ats-1 into the cytoplasm, which directly binds BECN1 and induces autophagosome formation (Niu et al., 2012; Fig. 3). The induced autophagosomes are directed to *A. phagocytophilum* vacuoles, where they fuse and deliver their autophagic cargo. Thereby, *A. phagocytophilum* acquires additional nutrients needed for its bacterial growth (Niu and Rikihisa, 2013).

Coxiella burnetii replicates in *Coxiella*-containing vacuoles (CCVs) that are also decorated with autophagy components. A screen of *C. burnetii* mutants to characterize genes required for CCV biogenesis identified the effector protein Cig2, which seems to enhance fusion of autophagosomes with the CCV (Newton et al., 2014; Fig. 3). In the proposed model, Cig2 promotes fusion of the CCV with autophagosomes by continuously maintaining LC3 on the CCV membrane. Maintaining LC3 delays autophagosome maturation and promotes autophagosome fusion with other phagosomes and CCVs (Newton et al., 2014). This process may also be supported by another *Coxiella* effector protein, CvpB, which binds PI3P and perturbs the activity of the phosphatidylinositol 5-kinase PIKfyve, thereby enriching PI3P on CCV membranes (Martinez et al., 2016; Fig. 3). Increased levels of PI3P at CCVs promote the recruitment of autophagosomes and CCV homotypic fusion.

Bacterial modulation of mTOR signaling

One central regulator of cellular metabolism is mechanistic target of rapamycin (mTOR), which is a core component of two complexes: mTORC1 (containing the protein Raptor), mediating cellular homeostasis, and mTORC2 (containing the protein Rictor; Martin et al., 2012). When nutrients are plentiful, mTORC1 is active and phosphorylates components of the autophagy induction machinery, resulting in autophagy repression (Zoncu et al., 2011; Fig. 4). *S. typhimurium* actively induces mTOR activation by recruiting the focal adhesion kinase (FAK) to the surface of *Salmonella*-containing vacuoles. FAK activation leads to the AKT-dependent activation of mTOR, which in turn inhibits autophagy (Owen et al., 2014; Fig. 4).

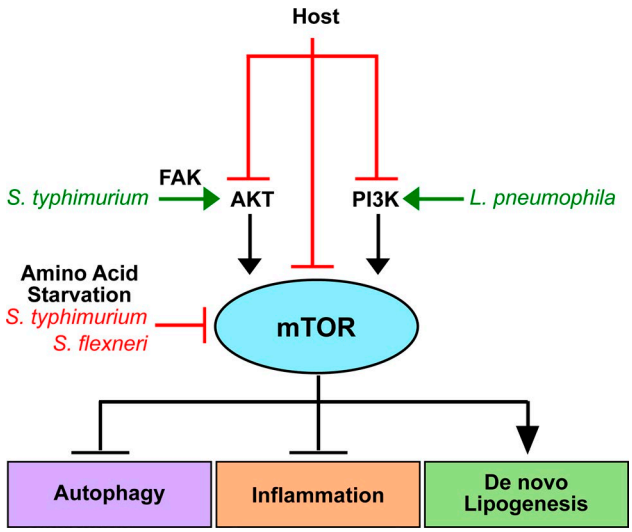

Figure 4. **Modulation of mTOR signaling during infection.** During steady-state conditions, mTOR is a negative regulator of autophagy and inflammation, as well as a positive regulator of de novo lipogenesis (black lines). (A) During infection, the host will inhibit mTOR signaling to activate autophagy and inflammation and inhibit de novo lipogenesis. It does so by targeting mTOR and positive regulators of mTOR (i.e., AKT and PI3K) for proteasomal degradation. mTOR is also inhibited by amino acid starvation that occurs during infection by certain pathogens (e.g., *S. typhimurium and S. flexneri*). Some bacterial pathogens (e.g., *S. typhimurium* or *L. pneumophila*) activate positive regulators of mTOR signaling to counteract these host-driven effects and thereby improve their intracellular housing capacity. In the case of *S. typhimurium*, AKT is activated by FAK that is recruited to the surface of *Salmonella*-containing vacuoles.

However, infection with pathogenic bacteria normally leads to a down-regulation of mTOR activity. For example, both *S. typhimurium* and *S. flexneri* induce amino acid starvation in infected epithelial cells, which results in mTOR inhibition (Tattoli et al., 2012). During infection with *L. pneumophila*, the mTOR inhibition is mediated by a host-driven ubiquitination of positive mTOR regulators (i.e., PI3K and AKT), as well as by the ubiquitination of mTOR itself (Fig. 4). The down-regulation of mTOR by the host is dependent on the TLR adapter protein MyD88, and results in an increased expression of certain proinflammatory cytokines (e.g., IL-6 and IL-1β) and decreased expression of anti-inflammatory cytokines (e.g., IL-10; Ivanov and Roy, 2013; Abshire et al., 2016). Interestingly, mTOR inhibition is counteracted by yet unidentified, secreted *L. pneumophila* effector proteins that activate mTOR via PI3K (Abshire et al., 2016). Because mTOR also controls host lipogenesis, its down-regulation during *L. pneumophila* infection leads to destabilized *Legionella*-containing vacuoles (LCVs); however, effectors that activate mTOR to increase host lipogenesis would favor *L. pneumophila* replication by promoting the expansion of the LCV (Abshire et al., 2016). This fine-tuning of mTOR signaling exemplifies the complex network of interactions that pathogens face when they invade a mammalian cell (Fig. 4). Furthermore, it illustrates the need of tailoring the response of

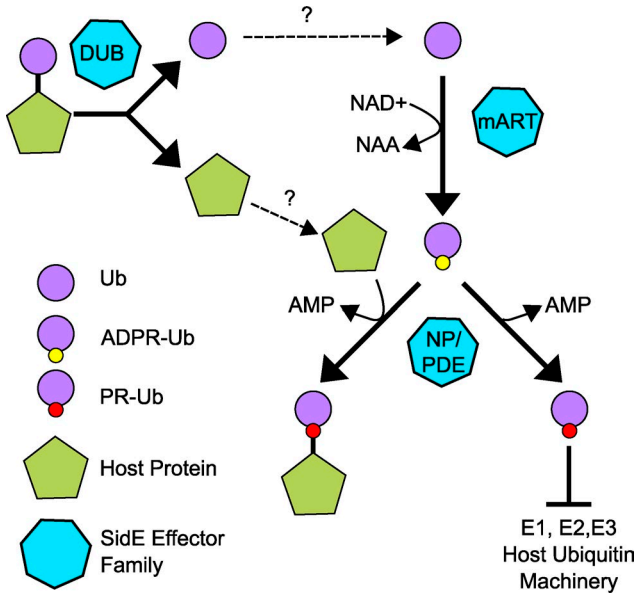

Figure 5. **Ubiquitination of host proteins by *Legionella* SidE effector family.** The SidE family of effectors from *L. pneumophila* modify host ubiquitin and ubiquitinate host proteins using a novel catalytic mechanism. The DUB domain of SidE effectors does not interfere with SidE-mediated ubiquitination, but instead, removes ubiquitin imparted by the canonical ubiquitination machinery of the host. It is unclear whether the DUB activity acts to generate a pool of ubiquitin and/or a pool of host target protein substrates for SidE effectors. The mono-ADP-ribosyltransferase (mART) domain uses NAD^+ to attach a phosphoribose moiety to arginine 42 of host ubiquitin, which generates ADP-ribosylated ubiquitin (ADPR-Ub) and nicotinamide (NAA). ADPR-Ub is further cleaved into phosphoribosylated ubiquitin (PR-Ub) and AMP by the nucleotidase/phosphohydrolase/phosphodiesterase (NP/PDE) domain. PR-Ub is covalently attached to host proteins via a noncanonical serine-linked phosphodiester bond. This novel ubiquitination mechanism does not require E1, E2, or E3 enzymes or ATP from the host. The generated pool of PR-Ub also disrupts canonical host ubiquitination machinery.

the host cell by activating and suppressing specific pathways at the same time to establish successful infection cycles.

Bacterial manipulation of host ubiquitination pathways

Ubiquitination is an important regulatory mechanism for cell signaling pathways. The covalent attachment of ubiquitin to a protein substrate is canonically an ATP-dependent enzymatic cascade involving three enzymes that activate ubiquitin (E1), conjugate ubiquitin (E2), and ligate ubiquitin (E3; Haglund and Dikic, 2005). One of the reasons that ubiquitin is such a powerful signaling molecule is that ubiquitin itself can be ubiquitinated at seven distinct lysine residues to form linear or branched chains. Hence, both the degree and the linkage of ubiquitination influence the fate of the substrate. Poly-ubiquitination on lysine 48 (UbK48) typically leads to proteasomal degradation of the substrate, whereas poly-ubiquitination on lysine 63 (UbK63) is associated with cell signaling. Typically, mono-ubiquitination of substrates is also associated with cell signaling (Haglund and Dikic, 2005). Deubiquitinases (DUBs) remove ubiquitin modifications and can act in a linkage-specific manner to reverse the fate of a ubiquitinated protein (Yau and Rape, 2016). Effectors secreted by intracellular bacteria can modulate the host ubiquitination pathway by mimicking host ubiquitination enzymes, such as E3 ubiquitin ligases and DUBs (Zhou and Zhu, 2015). Here we highlight a recently discovered, novel ubiquitination mechanism used by *L. pneumophila* to modulate host signaling pathways.

Several groups have characterized a set of effector enzymes from *L. pneumophila* that catalyze a novel ubiquitination mechanism to modulate host signaling pathways (Sheedlo et al., 2015; Bhogaraju et al., 2016; Qiu et al., 2016; Kotewicz et al., 2017; Table 5). The SidE effector family (i.e., SidE, SdeA, SdeB, and SdeC) is important for the intracellular replication of *L. pneumophila* in amoeba (Qiu et al., 2016). Unlike canonical ubiquitination pathways, where E1, E2, and E3 enzymes use ATP to ubiquitinate lysine residues of their target substrate, members of the SidE family are unique in that they possess domains that confer multiple enzymatic functions used for ubiquitination into a single effector and do not require ATP (Fig. 5). Instead, a mono-ADP-ribosyltransferase domain allows members of the SidE effectors to use NAD^+ to posttranslationally modify host ubiquitin with a phosphoribose moiety on arginine 42 to generate ADP-ribosylated ubiquitin (ADPR-Ub; Qiu et al., 2016). The collective action of the a nucleotidase/phosphohydrolase/phosphodiesterase domain then cleaves ADPR-Ub into AMP and a phosphoribosylated ubiquitin (PR-Ub) and covalently attaches PR-Ub to host proteins via a noncanonical serine-linked phosphodiester bond (Bhogaraju et al., 2016; Qiu et al., 2016; Kotewicz et al., 2017). Members of the SidE effector family also contain a DUB domain that can remove ubiquitin modifications imparted by the canonical host ubiquitination machinery without interfering with the SidE-mediated ubiquitination (Sheedlo et al., 2015). In addition, the DUB activity of SidE effectors reduces the level of ubiquitination on the surface of the LCV (Sheedlo et al., 2015). Moreover, the activity of the SidE effector family also generates a pool of PR-Ub that impairs conventional host ubiquitination pathways by interfering with host E1 and E2 enzymes when overexpressed in either mammalian or yeast cells (Bhogaraju et al., 2016; Kotewicz et al., 2017). Overexpressing members of the SidE effector family (i.e., SdeA) also interferes

Table 5. **Glossary of effectors: Bacterial manipulation of host ubiquitination pathways**

Bacteria	Effector	Host target	Target pathways	Outcome	Mode of action	Reference
L. pneumophila	SidE effector family	Rabs; Reticulon 4	UbDCP[a]	Inhibition of mitophagy,[b] innate immunity,[b] proteasomal degradation,[b] membrane trafficking, ER structure	Ubiquitin ligase/DUB	Sheedlo et al., 2015; Bhogaraju et al., 2016; Qiu et al., 2016; Kotewicz et al., 2017
L. pneumophila	SidJ	SidE-ubiquitinated substrates; host-ubiquitinated substrates	UbDCP	Removes ubiquitination left by the host as well as from SidE effector family	DUB	Qiu et al., 2017

[a]UbDCP, ubiquitination-dependent cellular processes.
[b]Results from overexpression of SidE effector family member.

with several ubiquitin-regulated processes in the host, including mitophagy, immunity (i.e., TNF-induced NF-κB nuclear translocation), and proteasomal degradation (i.e., constitutive degradation of hypoxia inducing factor 1α (Bhogaraju et al., 2016). Furthermore, members of the SidE effector family facilitate LCV biogenesis by targeting both Rabs and the ER-resident reticulon family of proteins to modulate host membrane trafficking and ER structure, respectively (Bhogaraju et al., 2016; Qiu et al., 2016; Kotewicz et al., 2017). Remarkably, *L. pneumophila* also secretes a DUB effector, SidJ, which reverses the ubiquitin modification imparted by members of the SidE effector family, thereby ensuring that the effects of these ubiquitin modifications are temporally regulated (Qiu et al., 2017). SidJ removes not only PR-Ub moieties left by the SidE effector family, but also ubiquitin modifications left by the canonical mammalian ubiquitination machinery (Qiu et al., 2017). Overall, this novel ubiquitination mechanism represents a potent weapon in the arsenal of *Legionella* effectors that can be deployed with temporal precision to manipulate host cell signaling pathways.

Conclusions and future perspectives

Intracellular bacteria are challenged with maintaining a delicate balance of weakening their host to prevent clearance, while at the same time keeping their host healthy enough to establish a suitable niche for intracellular replication. They modulate host intracellular signaling pathways in their favor to both shuttle resources to their replicative vacuoles and disarm host defense mechanisms. Dissecting the molecular mechanisms of this never-ending battle has the potential to strongly impact our understanding of host evasion by pathogens, as well as greatly expand our knowledge of host defense pathways.

We have described a diverse array of bacterial effectors that have been shaped by evolution to mimic host enzymes, as well as effectors that perform novel enzymatic activities, to modulate innate immunity, the UPR, and autophagic signaling pathways of their hosts. Using intracellular bacteria to probe the many ways in which kinase cascades, ubiquitin cascades, and epigenetic gene regulation can be modulated will serve as a powerful tool moving forward as the field continues to untangle these signaling pathways. The complexity and the precise coordination orchestrated by bacteria to modulate host cell signaling are astonishing. We have explored how bacterial pathogens can target different pathways simultaneously or even one pathway with several effectors. Moreover, different bacteria have developed remarkably innovative and individualized strategies to target the same host cell signaling pathways. This shows the overwhelming variety in bacterial effectors that have been discovered to date, and highlights the importance of studying bacterial effector proteins to reveal novel enzymatic activities that can regulate host cell signaling. Furthermore, the crosstalk between the different signaling pathways is only just beginning to be elucidated and adds another layer of complexity.

Pathogens, in many ways, are nature's cell biologists. Research into how pathogens rewire host intracellular signaling pathways is central to understanding infectious diseases and comprises an exciting interdisciplinary field by combining microbiology, cell biology, biochemistry, and immunology. A better understanding of the survival strategies used by intracellular pathogens will prove useful for the rational design of novel approaches and therapies to fight infectious diseases and will provide a deeper insight into the inner workings of our own cells.

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