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,
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., 2012; 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 Mycobacterium 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

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**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 kinases | Belyi et al., 2006, 2008; Fontana et al., 2011, 2012 |
| *C. difficile*  | TcdA | Unknown | Unknown | Unknown | Enhanced immune response | Glucosyltransferase kinases | Cowardin et al., 2016 |
| *P. aeruginosa* | ToxA | Unknown | Protein translation | Enhanced immune response | AB exotoxin | Dunbar et al., 2012; McEwan et al., 2012 |
| *Y. pestis*     | YopJ | MAP2Ks | MAPK | Inhibits MAPK and NF-κB signaling | Serine/threonine acetyltransferase | Orth et al., 1999 |
| *S. typhimurium*| AvrA | MAP2Ks | MAPK | Inhibits apoptosis | Serine/threonine acetyltransferase | Mukherjee et al., 2006; Paquette et al., 2012 |
| *L. monocytogenes* | InhC | IKK | NF-κB | Reduces degradation of IκB | Binds IKK | Jones et al., 2008; Wu et al., 2013 |
| *S. flexneri*   | IpH1.4 | LUBAC | NF-κB | Inhibits the activation of IKK | E3 ubiquitin ligase | de Jong et al., 2016 |
| *S. flexneri*   | IpH2.5 | LUBAC | NF-κB | Inhibits the activation of IKK | E3 ubiquitin ligase | de Jong et al., 2016 |
| *S. flexneri*   | IpH9.8 | 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 | Ub1c13 (E2 ubiquitin-conjugating enzyme) | NF-κB | Inactivates Ub1c13 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 |

*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 *Listeria* (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 (Dolowski 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., cyclic GMP or Ca2+) 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 to block MAPK activation. YopJ also targets 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 (LUBAC) for proteasomal degradation and suppress the activation of NF-κB (de Jong et al., 2016). LUBAC is a multifaceted, host E3 ubiquitin ligase that normally activates IKK with methionine 1-linked linear ubiquitin chains (Walczak et al., 2012). Another *S. flexneri* effector, Ospl, 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         | Deyacylation 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 | MAPK            | Dephosphorylation of H3S10                   | Phosphothreonine lyase              | Li et al., 2007; Zhu et al., 2007; Haneda et al., 2012 |
| *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      | Histon H2B, H3, and H4 | Direct PTM | Transcriptional repression                 | Methyltransferase                  | Pennini et al., 2010        |
| *L. pneumophila Paris*   | RamA     | Histone H3  | Direct PTM      | Methylation of H3K14; transcriptional repression | Methyltransferase                  | Rolando et al., 2013        |
| *L. pneumophila Philadelphia IP02* | LegAS4 | Histone H3  | Direct PTM      | Methylation of H3K4; transcriptional activation of ribosomal RNA genes | Methyltransferase                  | Li et al., 2013             |
| *B. anthracis*           | BoSET    | Histone H1  | Direct PTM; NF-κB | Trimethylation of histone H1; transcriptional repression of NF-κB target genes | Methyltransferase                  | Mijitaba 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         |

*PTM, posttranslational modification.

*Extracellular pathogen.*
Histone modifications by bacterial virulence factors

MAPK-mediated innate immune signaling pathways also introduce or remove postranslational 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). Post-translational 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-kB 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 (Sanada 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-kB 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., cxc12 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 cytolsin pore-forming toxins that is shared by other bacterial pathogens. Remarkably, purified cholesterol-dependent cytolsin 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 (InIB), also induces deacetylation of histone 3 on lysine 18 (H3K18) by activating a host histone deacetylase, sir- tuin 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-kB regulated genes (e.g., IL-8) and attenuates the binding of NF-kB 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) effector and localizes to the host cell nucleus. Interestingly, NUE exhibits automethyltransfer 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...
Intracellular bacteria usurp host defenses

• Cornejo et al. 3937

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. 1D). 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. 1D). 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

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.

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 | Inhibition of XBP1u mRNA splicing | Unknown        | Hempstead and Isberg, 2015; Treacy-Abarca and Mukherjee, 2015 |
| L. pneumophila    | Lgt1, Lgt2, Lgt3 | Unknown | IRE1            | Activation of the UPR    | 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-xB and NF-kB 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 (XBPIu), resulting in spliced XBPI mRNA (XBPIs). XBPIs 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 (Iurlaru 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 is in 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 XBPIu 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 restructur-
In vivo studies show that necrosis is reduced and the survival of pups increases when mice are infected with the *B. abortus* vccC 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 (Martinson 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 posttranslationally modifying eif2, 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 effecter 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 effectors 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 Brunell, 2014).

In principle, xenophagy follows the basic steps of the autophagy pathway (Fig. 3), which can be induced by a variety...
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 sepsins 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         | 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 lipidded LC3          | Cysteine protease | Choy et al., 2012; Horenkamp et al., 2015; Yang et al., 2017 |
| C. burnetii              | 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; phosphatidylinositol S-kinase | Autophagy | Enhanced association of the autophagy machinery to CCVs; homotypic fusion of CCVs | Unknown         | Martinez et al., 2016 |

*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 sequestered by a T4SS and localizes to the autophagosome. RavZ extracts LC3-PE from the membrane, irreversibly cleaves lipitated 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, CvBp, 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).

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
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.

**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. The 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.

**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* | Inhibition of mitophagy, innate immunity, proteasomal degradation, 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                                    |

*UbDCP, ubiquitination-dependent cellular processes.
*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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