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Bacteria, the ER and the Unfolded Protein Response: Friends or Foes?

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Abstract

The Unfolded Protein Response (UPR) is a cytoprotective response aimed at restoring cellular homeostasis following physiological stress exerted on the endoplasmic reticulum (ER) that also invokes innate immune signaling in response to invading microorganisms. While the UPR is modulated by various viruses, recent evidence indicates that it also plays multiple roles during bacterial infections. In this Review, we describe how bacteria adapt to live in the ER and discuss the intricacies of bacterial interactions with the UPR, including how UPR subversion promotes the proliferation of intracellular bacterial pathogens and how the UPR contributes to innate immune responses against invading bacteria.

Bacterial pathogens with an intracellular life cycle have devised various strategies to subvert specific compartments within host cells and generate niches that ensure their survival, persistence and proliferation. Bacterial entry into eukaryotic cells generally results in bacteria residing within phagosomes, which are intracellular compartments dedicated to innate immune detection and degradation of incoming microorganisms, leading to antigen presentation and development of adaptive immunity. Despite these immune processes, bacteria entrapped within phagosomes can achieve intracellular survival by various means, including interference with phagosomal maturation to impair fusion with lysosomes, phagosomal disruption and release into the cytosol. Bacteria can also transform the original phagosome into an idiosyncratic vacuole that acquires functional properties of less antimicrobial intracellular compartments. For example, bacteria can modulate the phagosome to interact with the endoplasmic reticulum (ER), a large membrane-bound organelle that ensures biosynthesis of proteins, carbohydrates and lipids, and orchestrates their transport along the secretory pathway. The ER delivers these components to their destination compartments, which include the ER itself, the Golgi apparatus, the plasma membrane, the extracellular milieu, or the endocytic and autophagic pathways. Given its biosynthetic functions and role along the secretory pathway, the ER stands as a nutrient-rich intracellular location that is presumably devoid of bactericidal functions, such as antimicrobial peptides or hydrolytic enzymes, intuitively making it a suitable niche for the intracellular survival, persistence and proliferation of intracellular bacteria.

The ER plays crucial roles in cellular homeostasis by controlling processing and folding of secretory and membrane proteins. When protein folding requirements exceed the ER processing capacity, unfolded proteins accumulate, induce ER stress and trigger the unfolded protein response (UPR), an evolutionarily conserved cytoprotective signaling pathway. By inhibiting mRNA translation, increasing the ER protein folding capacity and ER-associated degradation (ERAD), the UPR serves to relieve physiological stress on the ER and maintain cellular homeostasis[1]. Failure to restore ER functions results in programmed cell death. In addition, the UPR triggers signal transduction events associated with innate immunity and host defense, linking this physiological response to detection of intracellular pathogens[2].

Viral infections have been long known to exert stress on the ER and induce the UPR due to their demand on protein synthesis, and several viruses modulate the UPR to ensure viral protein production, replication and cell survival[3] (Box 1). Similarly, bacterial proliferation in the ER likely causes physiological strain on this compartment that can result in ER stress and the induction of the UPR. In agreement with this scenario, recent evidence indicates important roles of the UPR in either promoting or counteracting intracellular proliferation of bacterial pathogens that subvert ER functions, and in sensing effects of bacterial protein delivery into cells. Here we will present and discuss recent findings that support the UPR as a key component of crosstalk between the ER, intracellular bacteria and their pathogenic activities, and how it may contribute to inflammatory and immune responses to intracellular bacteria.

The UPR: components and functions

Nearly one third of the eukaryotic proteome is synthesized in the endoplasmic reticulum (ER) and enters the secretory pathway. The ER is also the site for biosynthesis of membrane lipids and cholesterol. In the lumen of the ER, proteins fold into their correct conformation with the assistance of the protein glycosylation machinery and dedicated chaperones, such as BiP (also known as GRP78), calnexin and calreticulin[4]. Under certain stress conditions, such as elevated synthesis of secretory proteins, perturbations in calcium homeostasis and redox balance, the abundance of misfolded proteins exceeds the capacity of the protein folding machinery. In response to these perturbations, three sensors located in the ER membrane, inositol-requiring enzyme 1 (IRE1), double-stranded RNA-dependent protein kinase (PKR)-like ER kinase (PERK) and activating transcription factor 6 (ATF6), direct induction of the UPR[1,4,5] (Figure 1). Each of these proteins has an ER-targeting transmembrane domain that links a cytosolic effector domain and a domain localized to the lumen of the ER. While the precise molecular mechanisms leading to activation of these ER sensors are still unclear, experimental evidence supports two different inputs to this process. The first is direct binding of unfolded proteins to the lumenal domain. This interaction has been demonstrated for yeast Ire1, for which the dimerized protein was shown to form a polypeptide binding groove[6], and a similar structural feature has been identified in PERK[1]. A second input is binding of the ER-lumenal domains of IRE1, PERK and ATF6 by the ER chaperone BiP. As a result of ER stress conditions that increase the load of protein misfolding or misassembly of protein complexes, the cell's capacity for protein folding is exceeded, and BiP is recruited away from IRE1, PERK and ATF6 to assist in refolding

proteins within the ER, which correlates with activation of IRE1, PERK and ATF6. Additional signals emanating from the ER membrane, as well as the cytosol, have also been proposed to activate the UPR[1]. Activation of IRE1, PERK and ATF6 initiates signaling pathways of the UPR to restore homeostasis in the ER, via increasing the production of chaperones to assist in protein folding, arresting translation of proteins not involved in resolving ER stress, and degradation of terminally misfolded proteins via the ER-associated degradation (ERAD) pathway, which orchestrates their retrotranslocation through the ER membrane, polyubiquitination and targeting to the proteasome[7].

IRE1 is a multifunctional protein that possesses kinase and endonuclease activities. The activity of IRE1 is downmodulated by binding of BiP[8–10] and is stimulated by direct binding of unfolded proteins[2,6]. Upon activation, IRE1 aggregates and autophosphorylates, activating its endonuclease activity[11]. IRE1's site-specific endonuclease activity splices the mRNA encoding the transcription factor XBP1, which results in translation of an active protein[12]. Production of XBP1 directs the expression of ER-resident molecular chaperones and protein folding enzymes[13]. In addition to splicing the XBP1 transcript, IRE1 has a nonspecific endonuclease activity that is responsible for rapid degradation of ER membrane-associated mRNA by a process known as regulated IRE1-dependent decay (RIDD)[14,15]. This response reduces the load of protein entering the secretory pathway.

Like IRE1, PERK is also an ER stress-activated kinase. Upon activation by ER stress, PERK homodimerizes and transphosphorylates, which is accompanied by dissociation of BiP. Activated PERK can then phosphorylate its target, the translation initiation factor eIF2α[8,16]. Phosphorylation of eIF2α inhibits synthesis of secretory proteins by inhibiting the assembly of the 80S ribosome, thereby promoting cellular survival during ER stress[17]. At the same time, phosphorylated eIF2α directs translation of the mRNA encoding the transcription factor ATF4, which induces expression of UPR target genes involved in amino acid transport and oxidative stress resistance[17]. Under conditions of prolonged or severe ER stress that the UPR cannot resolve, ATF4 also increases the levels of the transcription factor CHOP, which upregulates genes involved in the induction of apoptotic cell death[18].

The third arm of the UPR is initiated by ATF6. Activation of ATF6 results in its translocation to the Golgi, where it is cleaved by the Golgi-resident proteases site-1 protease (S1P) and S2P[19]. The active amino terminus of ATF6 is translocated to the nucleus, where it binds the ER stress response element upstream of a subset of UPR genes to activate their transcription[20]. The set of ATF6-dependent genes overlaps partially with genes induced by IRE1α and PERK and includes genes with functions in protein folding, protein transport and lipid biosynthesis[20,21]. In addition, ATF6 can homodimerize with XBP1 to activate genes involved in ERAD[22].

One of the cellular responses downstream of the UPR is the induction of autophagy. Induction of the UPR increases membrane lipid biogenesis and triggers ER expansion via the XBP1 and ATF6 pathways, as a means to enhance ER function[23–25]. Selective autophagy of the ER (so-called reticulophagy or ER-phagy) aims to regulate ER expansion and promote restoration of cellular homeostasis upon resolution of ER stress. While induction of autophagy has been shown to promote survival of cancer cells[26], the effect of

ER stress-induced autophagy on cell survival appears to be context-dependent, as ER stress-induced autophagy led to death of primary cells[27]. Under conditions of strong or sustained ER stress, a failure of the cell to restore homeostasis triggers apoptotic cell death. While the precise pathways of apoptosis induced by ER stress are not known, the PERK-eIF2α-ATF4-CHOP pathway plays an important role by reversing translational arrest, increasing generation of reactive oxygen species and promoting calcium efflux from the ER. Together, these signals lead to cytochrome c release from mitochondria and loss of membrane potential, resulting in apoptosis[18].

In addition to autophagy, UPR signaling also interacts with innate immune signaling pathways leading to inflammation, as activation of IRE1 by ER stress can activate the MAP kinase JNK[28], a key player in the response to inflammatory stimuli[28](see below). Therefore, although residing in the ER might benefit the survival of intracellular bacteria, it is possible that UPR induction might serve as an innate immune mechanism against invading bacteria.

The ER, a safe niche for intracellular bacteria

Although the ER presumably embodies a hospitable, nutrient-rich organelle devoid of antimicrobial functions, only a handful of bacterial pathogens take advantage of its nature and functions to ensure their intracellular survival and proliferation. Additionally, a few other intracellular bacteria have developed tight interactions between their vacuole and this compartment. The first evidence of bacterial interactions with the ER came from the observations that *Legionella pneumophila* and *Brucella* spp. occupy ribosome-studded intracellular vacuoles[29–31], which were confirmed to be derived from the ER at both ultrastructural and functional levels[32–36]. Since these seminal demonstrations, *Legionella longbeachae*[37], *Chlamydia trachomatis*[38,39] and the *Chlamydia*-related bacterium *Simkania negevensis*[40] have also been shown to survive and replicate in organelles that closely interact with the ER.

Bacterial trafficking to the ER

The trafficking events of bacterial vacuoles leading to the biogenesis of *L. pneumophila* and *Brucella* spp. ER-derived organelles have been well characterized and both invoke subversion of early secretory vesicles, yet via distinct mechanisms (Figure 2). Upon phagocytosis by macrophages, plasma membrane-derived *Legionella*-containing vacuoles (LCV) avoid phagosomal maturation along the endocytic pathway. They instead intercept early secretory vesicles trafficking between the ER and the Golgi apparatus, which cover and subsequently fuse with LCVs, transforming them into vacuoles with functional features of early secretory compartments[34,36,41]. These events eventually allow LCVs to fuse with ER membranes[34,36,41] and become a replication-permissive organelle that expands upon bacterial proliferation. Similarly, *Brucella* spp. reside within a *Brucella*-containing vacuole (BCV) following phagocytic uptake or entry within non-professional phagocytes. Unlike *Legionella*, however, BCVs first undergo maturation along the endocytic pathway to become endosomal BCVs (eBCVs)[42], and partially acquire phagolysosomal properties[33,43–45]. This trafficking step is followed by interactions of BCVs with ER exit sites (ERES)[43,45], a sub-compartment of the ER dedicated to formation and budding of secretory vesicles prior to their transport to the Golgi apparatus. Sustained BCV-ERES

interactions lead to biogenesis of ER-derived vacuoles – named replicative BCVs (rBCVs) – through progressive exchange of endocytic membranes for ER-derived membranes[43]. rBCVs support bacterial replication, which is accompanied by further ER membrane accretion and a dramatic reorganization of the ER into rBCVs when bacterial proliferation is extensive[35].

The obligate intracellular bacteria *Chlamydia* spp. reside within a large vacuole, the *Chlamydia* inclusion, which intercepts exocytic traffic between the Golgi apparatus and the plasma membrane to acquire sphingolipids necessary for inclusion biogenesis and bacterial growth[46,47]. Recently, interactions between the *Chlamydia* inclusion and the ER have been revealed in the form of points of contact, or "synapses", between these compartments[38,39]. These synapses may deliver ER-derived material to the inclusion[39] and are important for inclusion biogenesis and bacterial growth[38,39]. Similarly, the *Chlamydia*-like organism *S. negevensis* generates a large networked vacuole that also harbors contact sites with the ER[40], suggesting a common strategy of ER subversion among *Chlamydiales*.

Mechanisms of biogenesis of ER-derived bacterial vacuoles

While the mechanisms underlying *Chlamydia* inclusion interactions with the ER remain to be characterized, extensive studies of the biogenesis of *L. pneumophila* and *Brucella abortus* ER-derived replicative organelles have provided useful insight into how these pathogens reach the secretory compartment and establish residency in the ER. Both pathogens express a Type IV secretion system (T4SS), the *Legionella* Dot/Icm and *Brucella* VirB T4SS (Box 2), which direct the intracellular fate of the bacteria by delivering effector proteins into infected cells that modulate host cell pathways. Concomitant with uptake, *L. pneumophila* delivers the Dot/Icm effector proteins DrrA/SidM, LepB and RalF, which coordinately act to mediate LCV targeting to the ER via the modulation of small GTPases that normally control early secretory vesicle transport (Figure 2). DrrA/SidM acts as a GDP/GTP exchange factor (GEF) that recruits and activates Rab1 on plasma membrane-derived LCVs to promote recruitment of early secretory vesicles[48,49]. This process is negatively regulated by LepB, a GTPase activating protein (GAP) that deactivates Rab1[50]. Fusion of secretory vesicles with LCVs involves DrrA-dependent unusual pairing of plasma membrane and ER-derived vesicle SNARE proteins[51,52], which allows direct fusions between a plasma membrane-derived vacuole and early secretory vesicles, thereby mediating LCV bypass of the endocytic pathway. Subsequent to LCV fusion with secretory vesicles, RalF acts as a GEF for ARF1 and promotes its recruitment to LCVs and fusion with ER membranes to generate an ER-derived *Legionella* replication-permissive organelle[41,53,54]. Hence, this pathogen uses an array of effector proteins that co-opt membrane trafficking processes between secretory compartments to promote its residence within an ER-derived organelle.

The role of the *Brucella* VirB T4SS in BCV conversion into an ER-derived organelle has been established based on the inability of *virB* mutants to undergo sustained interactions with ERES and fuse with the ER, which results in failure to replicate and killing within eBCVs[35,43,55]. Through acidification and presumably additional intravacuolar cues, eBCVs signal intracellular *Brucella* to induce expression of the VirB apparatus[44,45,56]. Unlike *Legionella*, very little is known about the effector proteins that the *Brucella* VirB T4SS

delivers during infection. The recent identification of 14 *Brucella* proteins delivered into host cells by the VirB apparatus[57–61] is an important step towards a molecular understanding of VirB functions, but it has yet to yield information on whether these effectors mediate rBCV biogenesis. Several VirB effectors, such as VceC, BspA, BspB, BspC, BspD and BspF, target compartments of the secretory pathway when expressed ectopically in mammalian cells[60,62], suggesting they may modulate specific functions associated with secretory transport. Accordingly, expression of BspA, BspB and BspF impairs secretory trafficking during *Brucella* infection[60], indicating that the bacterium employs Type IV secretion to modulate functions of the secretory pathway. Indeed, *Brucella* requires functional ERES and the small GTPase Sar1 – which controls COPII-dependent vesicular budding from ERES[63,64] – to generate rBCVs[43]. rBCV biogenesis also requires the small GTPase Rab2, which the *Brucella* effector RicA binds[58,65]. RicA specifically interacts with the GDP-bound form of Rab2 but does not exhibit any guanine nucleotide exchange factor (GEF) activity[58], so how this interaction modulates Rab2 function remains unclear. Unlike *Legionella*, ARF1-dependent vesicular trafficking between ERES and the Golgi apparatus is not required for rBCV biogenesis[43], highlighting mechanistic differences in how both pathogens reach the ER.

Bacteria and the UPR

While many of the mechanistic underpinnings of bacterial interactions with the ER remain to be understood, *Legionella* and *Brucella* spp. stand as model organisms to study bacterial exploitation of this compartment and its consequences. The rare utilization of the ER by bacterial pathogens for survival and replication raises the question of its actual permissiveness to infection. Much like many viruses need to modulate the UPR to ensure their replication while overwhelming the biosynthetic capacity of the ER (Box 1) and protozoan parasites modulate UPR pathways to promote their pathogenesis (Box 3), bacteria must be able to deal with the consequences of their proliferation within the ER. Although these consequences are unclear, bacterial replication within the ER may elicit the UPR, which could trigger sensing of bacterial pathogenic activities.

Bacterial induction of the UPR

Several examples support the notion that bacterial infections trigger ER stress and can be sensed via the UPR. The UPR is induced in macrophage-rich granulomatous lesions of mouse lungs infected with virulent *Mycobacterium tuberculosis*, where apoptotic events are detectable[66] (Table 1). UPR induction has also been correlated with *Helicobacter*-induced gastric carcinogenesis[67] and occurs in gastric epithelial cells via the action of the vacuolating cytotoxin VacA[68]. VacA intoxication activates PERK and IEF2α, resulting in CHOP induction, mitochondrial dysfunction and apoptosis[68] (Table 1). This highlights the role of ER stress in responses to infections and toxin activities.

Similarly, *in vitro* cellular models of infection have revealed UPR induction in macrophages and epithelial cells infected with either *Brucella melitensis* and *B. abortus*[62,69] or *Listeria monocytogenes*[70] (Table 1). The first demonstration of a role of components of the UPR in the *Brucella* intracellular cycle was the discovery that IRE1α is necessary for *B. abortus* intracellular growth, through an RNAi screen for ER-associated factors required for

bacterial replication in *Drosophila melanogaster* S2 cells[71]. Confirmed in mammalian cells, IRE1α dependency of *Brucella* replication suggests that the UPR is beneficial to the infection. However, depletion of neither ATF6 nor PERK reproduced the effect of IRE1α depletion[71], which made a role for a canonical UPR in *Brucella* replication less substantiated.

Recently, direct evidence that *B. melitensis in vitro* and *in vivo* infection of murine macrophages leads to induction of the three major signaling pathways of the UPR established that *Brucella* infection induces physiological ER stress[69]. Whether bacterial residence and proliferation in the ER is required for induction of the UPR was however not addressed in this study, and the fact that a VirB-deficient mutant (which does not reach the ER or replicate) also induced UPR signaling[69] suggests that this response may not be related to bacterial replication in the ER. It will also be important to address whether the three main UPR regulators are required for *Brucella*-induced UPR, or whether only IRE1α-dependent signaling contribute to bacterial replication. Counteracting the UPR response with the pharmacological chaperone tauroursodeoxycholic acid (TUDCA) reduces *B. melitensis* intracellular growth, suggesting that the UPR promotes bacterial intracellular growth[69]. Yet, obtaining more direct evidence will be important to further substantiate this beneficial role of the UPR.

L. monocytogenes induction of the UPR occurs via extracellular secretion of its cytolysin Listeriolysin O (LLO), which induces all arms of the UPR and leads to ER stress-specific apoptosis[70] (Table 1). The molecular mechanisms by which this toxin acts remain unclear, but may be related to LLO's ability to alter intracellular Ca^{2+} homeostasis via its pore-forming activity[72], as Ca^{2+} imbalance causes ER stress. Nonetheless, evidence that LLO treatment of mammalian cells itself triggers the UPR indicates that the UPR may be induced via external stimuli and may not be restricted to ER-dwelling pathogens.

Bacterial subversion of the UPR

While the occurrence of UPR induction upon bacterial infection is now supported by several examples, indications that bacteria can modulate this response are rather sparse. *L. pneumophila* recruits components of the ERAD on its vacuole to mediate turnover of bacterial effectors on the vacuolar surface[54], and the proteasome to generate amino-acids necessary for its intracellular growth[73], but it is undocumented whether *Legionella* modulates the UPR. By contrast, *S. negevensis* generates a replicative vacuole associated with induction of ER stress, which the bacterium subsequently down-regulates[40]. Additionally, *S. negevensis* prevents ER stress chemically-induced using tunicamycin or thapsigargin, as judged by decreased induction of BiP, impaired nuclear translocation of CHOP and reduced activation of eIF2α[40]. Both compounds induce the UPR by perturbing distinct ER functions - tunicamycin inhibits N-linked glycosylation in the ER and affects protein folding, while thapsigargin blocks the sarco-endoplasmic reticulum calcium ATPases (SERCA), leading to depletion of ER calcium stores - indicating that *S. negevensis* likely interfere with common UPR signaling pathways via mechanisms that are yet to be determined. Interestingly, high concentrations of these UPR inducers can overcome *S.*

negevensis-mediated inhibition of UPR and affect bacterial growth[40], suggesting that the bacterium actively modulates this response to benefit its intracellular proliferation.

Additional evidence that bacteria might modulate the UPR originates from the study of bacterial toxins (Table 1). Similar to the effect of LLO during *Listeria* infections[70], pore-forming toxin (PFT) intoxication of cells leads to induction of the UPR via the p38 MAP kinase pathway. Pore formation likely triggers p38 MAPK activation, which in turn induces the UPR via the IRE1-XBP-1 and ATF6, but not PERK, pathways, arguing that the UPR response to PFTs is specific and distinct from that induced by unfolded proteins[74]. UPR induction is protective against PFT[74], indicating that the UPR acts as a defense mechanism against cellular injury caused by bacterial toxins. Studies of the mode of action of the AB_5 cytotoxin family subtilase SubAB from Shiga-toxigenic *Escherichia coli* (STEC) have revealed that SubAB specifically cleaves the ER chaperone BiP[75,76], due to unique structural features of its active site. BiP cleavage triggers the IRE1, PERK and ATF6 signaling arms of the UPR to induce transient ER stress and causes cell cycle arrest[75,77]. Cell cycle arrest at the G0/G1 phase is thought to be a consequence of Cyclin D1 decrease via both inhibition of translation and proteasomal degradation[75]. This exemplifies the ability of pathogenic bacteria to induce the UPR via delivery of specific proteins that exhibit enzymatic activities directed towards host cell factors.

The observation that UPR induction following *Brucella* infection promotes intracellular growth also suggests that the UPR might be targeted by specific bacterial mechanisms to promote survival. UPR induction by *B. melitensis* depends upon the protein TcpB (Toll/Interleukin-1-like receptor domain-containing protein) – also called BtpA (*Brucella* TIR protein A)[61] – since a mutant lacking *tcpB* failed to induce the UPR and purified TcpB was sufficient to cause UPR induction[69]. TcpB has been shown to antagonize TLR2 and TLR4 signaling, via its interaction with MyD88 and the adapter protein Mal/TIRAP and inhibition of NF-κB activation[78–83], and also stabilizes microtubules[80], potentially affecting ER morphology. It will therefore be interesting to examine whether TcpB-mediated UPR induction results from any of these activities, or whether its UPR-inducing effect reflects yet another function. The recent demonstration that BtpA is translocated into host cells by *B. abortus*, possibly in a VirB-dependent manner[61], emphasizes the concept that bacterial pathogens may trigger ER stress via delivery of effectors or toxins, but also raises the question of VirB dependency of UPR induction by *Brucella*: if TcpB/BtpA is a VirB effector, one would expect that a VirB-deficient strain also fails to induce the UPR. Hence, further studies are needed to clarify these aspects of *Brucella* induction of the UPR.

UPR induction by *Brucella* may however result from more complex interactions with host cells involving several effectors. For example, the VirB T4SS effector VceC induces IRE1α-dependent signaling, leading to induction of the proinflammatory cytokines TNFα and interleukin (IL)-6[62]. VceC-mediated stimulation of UPR signaling possibly results from its interactions with and possible sequestration of the ER chaperone BiP, which could alter its ability to down-modulate IRE1α activation[9,10] (Figure 3). Additionally, individual expression of various *Brucella* effectors (BspC, BspG, BspH, BspI, BspK) also leads to induction of ER stress in HeLa cells, regardless of whether these proteins target the secretory compartment[60]. Interestingly, all these effectors induced BiP overexpression, but

not CHOP[60], suggesting that they modulate the IRE1 and ATF6, but not the PERK, pathways, potentially avoiding inhibition of translation, cell cycle arrest and cell death. Hence, our current knowledge of *Brucella* induction of the UPR suggests a multifactorial process that allows a finely tuned modulation of this pathway to the bacterium's benefit.

UPR and innate immune signaling

The co-occurrence of ER stress and inflammation in many chronic pathologies, including neurodegenerative disease, diabetes, obesity and inflammatory bowel disease, suggests interactions between the UPR and inflammation[84]. Indeed, proinflammatory cytokines can promote ER stress signaling via oxidative stress, which can alter redox homeostasis in the ER and lead to protein misfolding. In addition, nitric oxide (NO) produced during inflammation can perturb ER function via S-nitrosylation of protein disulfide isomerase (PDI), inhibiting its ability to catalyze disulfide bond formation during protein folding[85]. Further, enhancement of proinflammatory cytokine responses by the IRE1-XBP1 pathway in response to TLR2 and TLR4 ligands suggests additional points of intersection between innate immune and ER stress signaling pathways[86].

Conversely, ER stress can activate proinflammatory signaling pathways via multiple mechanisms (Figure 3). Overexpression of viral proteins in the ER has long been known to activate NF-κB and AP-1 transcription factors, which induce expression of proinflammatory cytokines such as IL-6[87,88]. One of the pathways linking ER stress to proinflammatory cytokine expression is dependent on IRE1's kinase activity. Activated IRE1 forms a complex with TRAF2, that activates JNK[28], an upstream signaling molecule leading to AP-1 activation[28]. In the TNF receptor signaling pathway, activated TRAF2 can also recruit IκB kinases, leading to their activation, which suggests that recruitment of TRAF2 to the ER membrane by IRE1 activation during the UPR may also promote activation of NF-κB. IRE1-dependent mRNA decay (RIDD)[15] can also activate innate immune responses, via generation of immunostimulatory mRNA fragments in the cytosol[2]. Similar to fragments generated by the antiviral endoribonuclease RNase L[89,90], these mRNA fragments generated by RIDD can be detected by the innate immune receptor RIG-I to activate NF-κB and IRF-3, driving expression of proinflammatory cytokines and interferon beta (IFNβ)[2,89,91]. IRE1's endonuclease activity also activates XBP-1, which has been shown to promote transcription of IFNβ via binding of an upstream enhancer, as well as promoting transcription of IL-6 and TNFα[86,92]. In addition to the NF-κB pathway, under conditions of irremediable ER stress, IRE1 can also activate NLRP3 inflammasomes in response to redox stress via thioredoxin-interacting protein (TXNIP), leading to IL-1β activation and pyroptotic cell death[93–95].

The PERK-eIF2-ATF4-CHOP arm of the UPR, which mediates inhibition of translation, interacts with innate immune signaling via activation of NF-κB. Since IκB has a short half-life, translational inhibition by this pathway results in more rapid turnover of IκB and, consequently, activation of NF-κB (Figure 3). ATF6 also plays a role in activation of NF-κB in the context of cellular intoxication by Subtilase cytotoxin, however the precise mechanism for this is not known[96]. An ATF6-like transcription factor, CREBH, is activated by ER stress in a manner similar to ATF6 requiring cleavage by S1P and S2P, and plays a

role in innate immunity by activating hepcidin expression, thereby limiting iron availability to infecting bacteria[97,98]. It is therefore likely that depending on the cellular and tissue context of infection, different combinations of these pathways may play a role in induction of innate immune responses by ER stress.

Studies of viral infection have revealed that hijacking of ER functions for production of viral proteins can trigger innate immune responses, however several viruses are able to exploit the resulting unfolded protein response to promote their replication (Box 1)[3]. Therefore, it is conceivable that subversion of ER function for intracellular replication of bacterial pathogens such as *Legionella*, *Brucella*, *Simkania* or *Chlamydia* may also activate a subset of these surveillance pathways.

Brucella spp. are an excellent model to study the intersection of the UPR and innate immunity, because they signal only weakly through TLRs that detect bacteria, such as TLR4 and TLR2[99,100]. Further, in a mouse model of infection, innate immune responses to *Brucella* are highly dependent on function of its VirB T4SS[101]. In infected macrophages, the IRE1 arm of the UPR appears to play a key role in the innate immune response to the T4SS, since silencing of IRE1 dampened production of inflammatory cytokines by infected cells. VceC, a T4SS effector protein that localizes to the ER, can activate IRE1-dependent secretion of IL-6 and TNFα[62]. As described above, the newly-identified T4SS substrates BspC, BspG, BspH, BspI, and BspK, can also activate the IRE1 pathway in HeLa cells; therefore it will be interesting to determine whether they synergize with VceC in inducing expression of proinflammatory cytokines during infection[60]. Further, it is not known how BtpA, recently shown to induce all three pathways of the UPR, influences UPR-induced inflammation[69]. However, induction of UPR-dependent inflammation during *Brucella* infection may underline some similarities in the host response between *Brucella* and viral infections with regard to their gene expression profiles *in vivo*[101,102].

Bacterial toxins can also induce UPR-dependent inflammation (Table 1). Some toxins, such as Shiga toxin and cholera toxin, reach their cytosolic targets via cellular uptake and retrotranslocation to the ER, where they fold before reaching their targets in the cell cytosol. Localization of an enzymatically inactive A subunit of cholera toxin (CTA) to the ER was recently found to activate NF-κB via an IRE1-dependent pathway in intestinal epithelial cells, via binding of unfolded CTA to IRE1[2] (Figure 3). Interestingly, the pathway leading to NF-κB activation by CTA involves activation of RIDD and sensing of the resulting mRNA fragments via a RIG-I/MAVS-dependent pathway. The A subunit of Shiga toxin, which triggers death of intoxicated cells via the UPR[103], is also able to activate IRE1 and induce NF-kB activation, likely via a mechanism similar to that identified for CTA[2]. Activation of immune cells, particularly T cells, by Shiga toxins has been postulated to play a role in disease progression of hemolytic-uremic syndrome caused by Shiga toxin-producing *E. coli* (STEC), raising the possibility of a connection between UPR activation in intoxicated cells and systemic inflammatory pathology[105].

As mentioned above, cleavage of BiP by Subtilase cytotoxin of STEC leads to induction of the three branches of the UPR, and consequently to a transient activation of NF-κB[96,106]. However, at subcytotoxic doses, pre-stimulation of cells with subtilase cytotoxin was shown

to blunt NF-κB activation induced by heterologous stimuli such as TLR4 agonists[107]. Therefore, the effect of this toxin on inflammatory responses during an infection may depend on the toxin dose in the context of an infection. The above examples illustrate that disruption of ER integrity can serve as a pathogenic pattern of infection that can be induced both by pathogens that target the ER directly to promote infection and pathogens that, via disruption of other cellular processes, perturb ER function. Further, in the context of additional infection-related signals, including TLR ligands[86] and proinflammatory cytokines[5], cellular surveillance of ER function is heightened to generate a more rapid innate immune response to ER stress.

The UPR – friend or foe?

While a number of bacterial pathogens can induce the UPR during infection, it is not clear in each case whether this response benefits the host or the pathogen. Modulation of ER function during infection by intracellular bacteria can promote bacterial infection by providing a replicative niche, but at the same time the resulting disruption of the secretory pathway can provide a pattern of pathogenesis that aids the innate immune system in recognizing intracellular infection and in mounting an appropriate defense. However, considering the more rapid evolution of bacterial pathogens compared to their hosts, it is likely that bacteria have evolved to modulate the UPR to their advantage during infection. Given the multitude of virulence genes expressed by bacterial pathogens that replicate in ER-associated cellular compartments, future work is likely to reveal to what extent viruses and bacteria use shared strategies to exploit this intracellular niche to promote their replication, as well as which novel mechanisms are employed by bacteria to manipulate signaling pathways emanating from the ER. Further, since the UPR has been targeted therapeutically in inflammatory disease and cancer[108], it would be intriguing to explore this avenue as an adjunct for treatment of intracellular bacterial infection, such as tuberculosis and brucellosis, which currently require long antibiotic treatment regimens. Given the central importance of the UPR in a multitude of disease processes, insights from this area of study are likely to lead to an improved understanding of the links between ER stress signaling pathways and pathology, not only in the context of infection, but also of neurodegenerative disease, diabetes, obesity, inflammatory bowel disease, and cancer.

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Box 1

Viruses and the UPR

Viral replication co-opts ER functions for production of viral glycoproteins, leading to induction of the UPR[109]. Since downstream effects of UPR activation including translational attenuation, ERAD and cell death can inhibit viral protein production, viruses express mechanisms of manipulation and avoidance of the UPR to replicate successfully. While the exact mechanism for this is unknown for most viruses, some of the viral proteins involved have been identified. For example, cytomegaloviruses (CMV) can both induce and modulate the UPR[110–112]. Induction of the UPR by human CMV protein US11 has been proposed to lead to degradation of MHC Class I, a mechanism that may promote chronic infection[112]. Further, HCMV protein UL50 and its murine CMV homolog M50, can both downregulate IRE1 to suppress the UPR[111]. Hepatitis C virus also downregulates the IRE1-XBP1 pathway to promote viral replication[113,114]. Rotavirus can modulate the UPR via sequestration of UPR components[115,116]. Since bacteria that replicate within the ER face a similar environment, they may share some of these strategies for modulation of the UPR with viral pathogens, however additional studies are needed to test this idea. Further, similar to what has been proposed for *Brucella abortus* infection[62], stimulation of the UPR during viral infection may serve as a pathogen-associated pattern that activates antiviral innate immunity[117].

Box 2

Type IV secretion systems in pathogenic bacteria

Type IV secretion systems (T4SS) are multi-protein complexes organized in ATP-powered, membrane spanning machineries that deliver proteins, protein-protein or nucleoprotein complexes into either prokaryotic or eukaryotic cells. Expressed in both Gram-negative and Gram-positive bacteria, they fulfill a range of functions including DNA transfer and effector protein delivery into target cells, therefore playing important roles in transfer of antibiotic resistance traits via bacterial conjugation, or in virulence mechanisms of pathogenic bacteria[118]. Based on phylogenetic relationships with T4SS harbored by the canonical conjugative plasmids, T4SS have been classified in subfamilies (Type IVA and Type IVB) that are represented in pathogenic bacteria by the VirB and Dot/Icm systems, respectively (reviewed in[119]). Many pathogenic bacteria express T4SS to mediate their interactions with host cells. These include *Agrobacterium tumefaciens*, *Helicobacter pylori*, *Bordetella pertussis*, *Rickettsia* spp., *Brucella* spp. and *Bartonella* spp., which encode Type IVA VirB T4SSs that deliver DNA-protein, multi-subunit protein toxin and proteins into eukaryotic cells, and the intracellular pathogens *Legionella pneumophila* and *Coxiella burnetii*, which express Type IVB Dot/Icm T4SSs that deliver effector protein into target cells[120]. T4SSs such as the *Brucella* VirB apparatus[121] and the *Legionella* and *Coxiella* Dot/Icm systems[122] are essential to the survival and proliferation of these intracellular pathogens, by translocating into various host cell compartments arrays of effector molecules that take control of host functions associated with the biogenesis and trafficking of the bacterial vacuole. As such, they constitute key virulence factors of many pathogenic bacteria.

Box 3

Toxoplasma gondii and the UPR

The Apicomplexan protozoan parasite *Toxoplasma gondii* occupies a parasitophorous vacuole that displays close interactions with the ER[123,124], suggesting it may influence functions of this compartment. Consistently, recent evidence indicates that apoptosis of placental trophoblasts and neural stem cells infected with *T. gondii* involves activation of ER stress pathways[125,126], via induction of CHOP, caspase 12 and the JNK pathway. Trophoblast apoptosis is initiated by oxidative stress following reactive oxygen species (ROS) production, while cell death stimuli in neural stem cells are unknown, yet these findings highlight a UPR response to *T. gondii* infection. Whether the UPR is actively modulated by *T. gondii* or an indirect response to cellular effects caused by the parasite needs further clarification. Yet, the demonstration that the *T. gondii* rhoptry protein ROP18 destabilizes the UPR transducer ATF6β to promote its pathogenesis[127] strongly suggests that this pathogen also manipulates UPR signaling to its benefit.

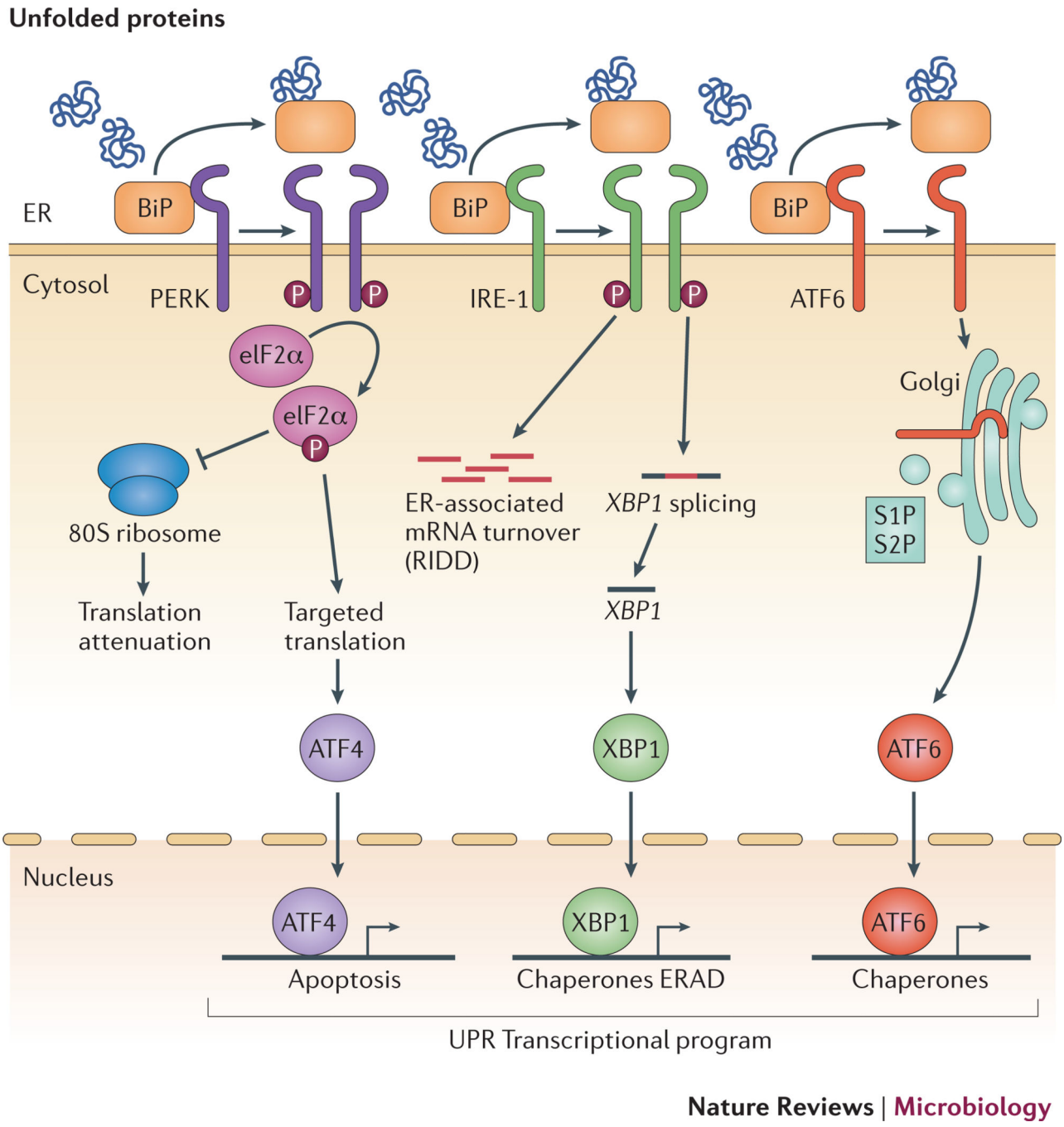

Figure 1. Cellular responses to endoplasmic reticulum stress

In response to increased in unfolded proteins in the ER lumen, three sensors located in the ER membrane, PERK, IRE1 and ATF6 activate the unfolded protein response. Under homeostatic conditions, PERK, IRE1 and ATF6 are bound by BiP, which suppresses their activity. In response to ER stress, BiP is recruited away from PERK, IRE1 and ATF6 to promote protein folding, which leads to activation of these ER stress sensors. PERK is a kinase, that auto-activates, dimerizes and phosphorylates its target eIF2, thereby preventing assembly of functional 80S ribosomes. Further, eIF2 directs selective translation of the

transcription factor ATF4, which activates transcription of genes involved in the unfolded protein response. IRE1 is a bifunctional enzyme with kinase and endonuclease activity. In response to ER stress, IRE1 auto-phosphorylates via its kinase domain, which leads to dimerization and activation of its endonuclease activity. The endonuclease function of IRE1 degrades mRNA at the ER to decrease protein biosynthesis and also splices the transcript encoding XBP1, a second transcription factor directing expression of genes involved in restoring cellular homeostasis. ATF6 is a transcription factor that is anchored in the ER membrane, but in response to ER stress, it translocates to the Golgi, where the transcription factor domain is released from the membrane by sequential action of Site 1 (S1) and Site 2 (S2) proteases, allowing its translocation to the nuclease to activate transcription of UPR target genes. ATF4, XBP1 and ATF6 direct a transcriptional program that upregulates chaperones, components of the ERAD pathway, and factors involved in autophagy and apoptosis that act to restore cellular homeostasis or if the disruption to ER function cannot be resolved, initiate programmed cell death.

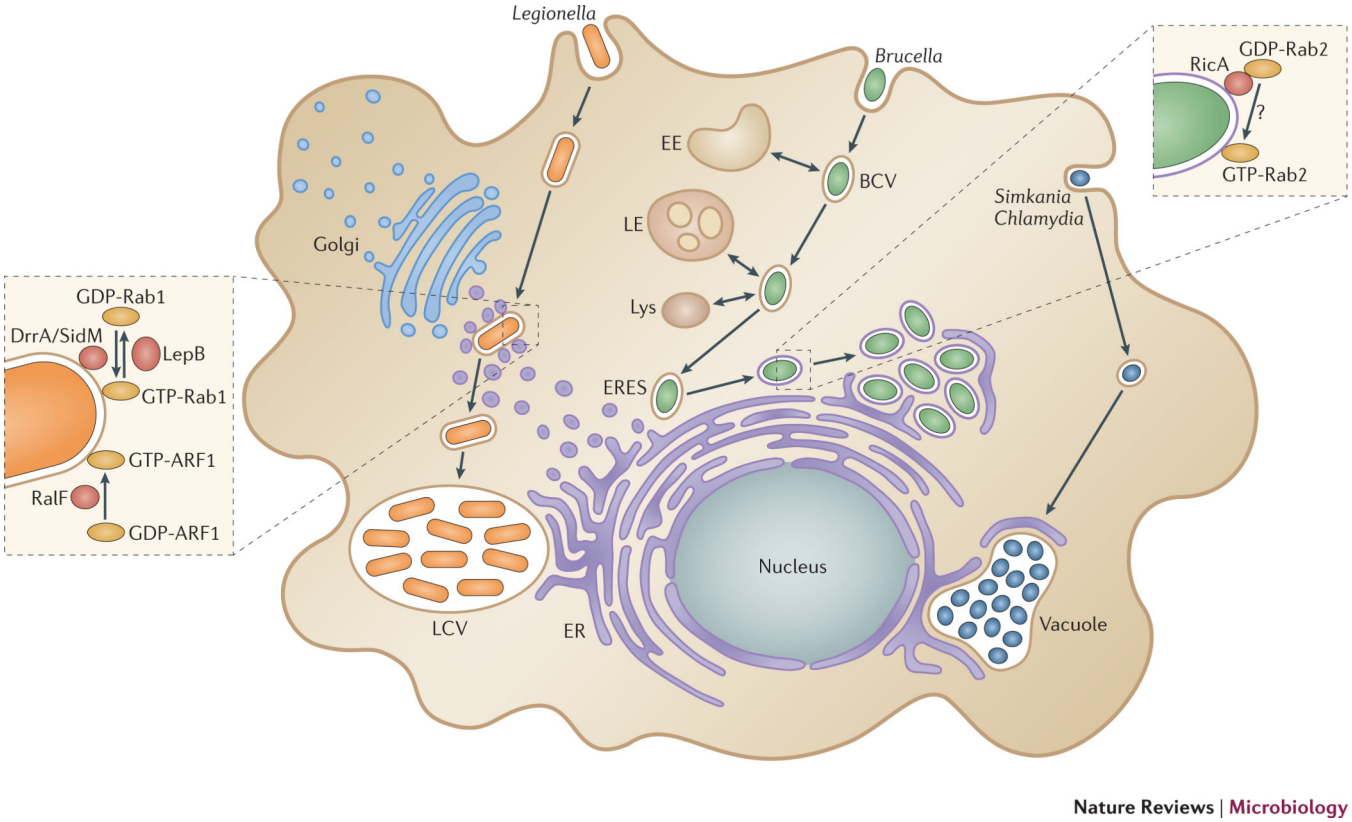

Figure 2. Trafficking and biogenesis of ER-associated replicative organelles by bacterial pathogens

Legionella pneumophila recruits early secretory vesicles to its plasma membrane-derived phagosome via delivery of the Dot/Icm Type IV secretion system effectors DrrA/SidM, LepB and RalF. These effectors modulate activities of the small GTPases Rab1 and ARF1 on the *Legionella* phagosome (see inset) to mediate bypass of the endocytic pathway and promote fusion of the *Legionella* phagosome with the ER and biogenesis of an ER-derived replicative vacuole. *Brucella* spp. reside within a vacuole (BCV) that traffics along the endocytic pathway, then is redirected to ER exit sites (ERES) and fuses with the ER via the action of VirB Type IV effector proteins and the small GTPases Sar1 and Rab2. The *Brucella* effector RicA binds GDP-bound Rab2 in an unknown manner and is required for accumulation of activated, GTP-bound Rab2 on BCVs (see inset). Chlamydia spp. and *Simkania* control the traffic and maturation of their original vacuole into a large inclusion that physically interacts with the ER at specific contact points (synapses).

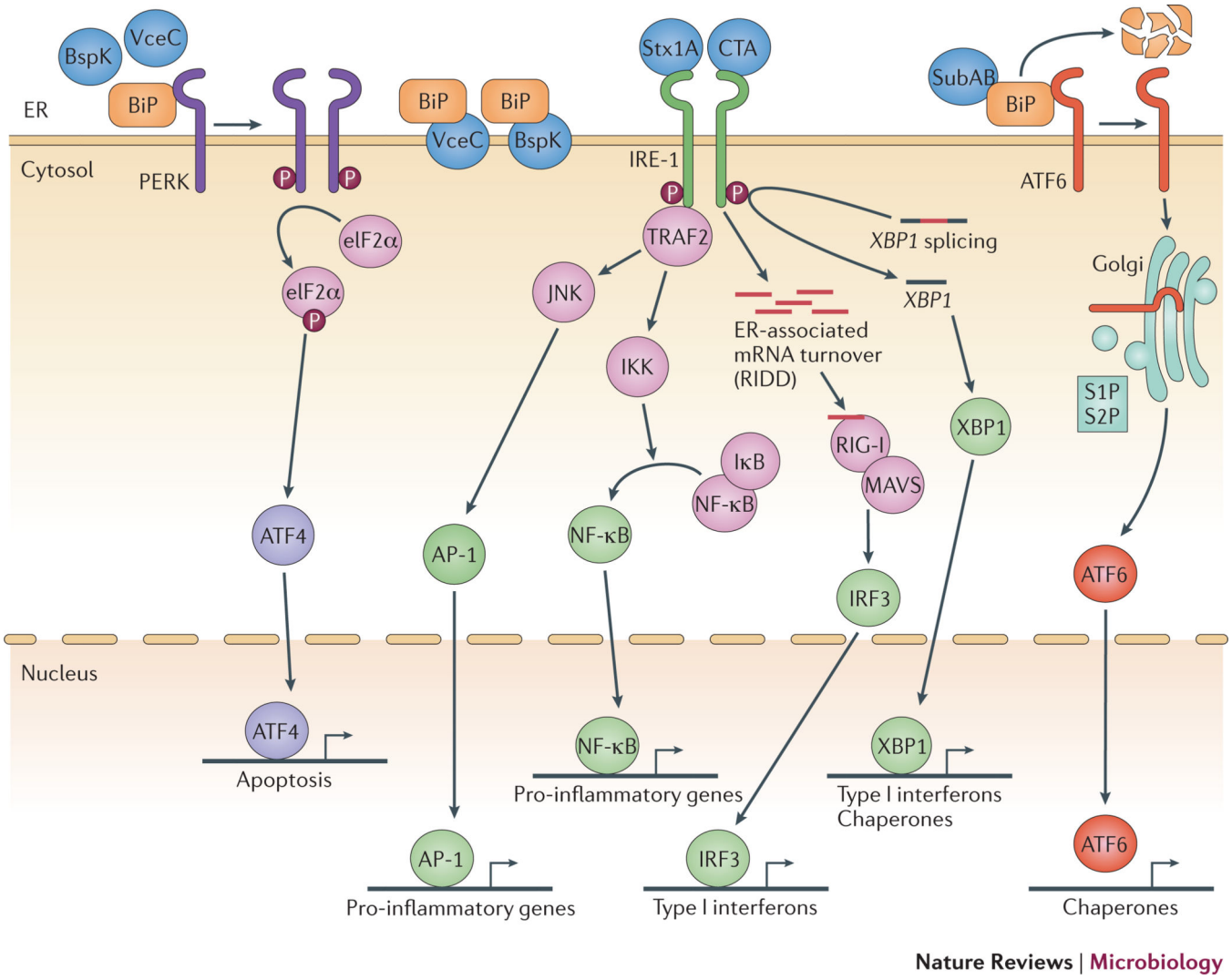

Figure 3. Induction of the UPR and inflammation by bacterial virulence factors

Both AB-type toxins and T4SS effectors have been implicated in activation of the UPR and subsequent inflammation. The A subunit of CTX binds IRE1α and activates its endonuclease activity, leading to activation of Type I interferon and NF-κB, and Shiga toxin A subunit appears to elicit the same pathway[2]. Subtilase cytotoxin also localizes to the ER and elicits the UPR by cleavage of the chaperone BiP[76], leading to activation of the UPR pathways and to NF-κB-dependent proinflammatory cytokine production. Two *B. abortus* T4SS effectors, VceC and BspK, target the ER, where VceC has been shown to bind to BiP. This binding may inhibit the interaction of BiP with PERK, IRE1α and ATF6, thereby promoting their activation. IRE1α interacts with TRAF2, leading to NF-κB activation[62].[60,6970]

Table 1

Bacteria and bacterial products that modulate the UPR.

Bacteria	Toxin or effector	Effect	Mode of action	Reference
Mycobacterium tuberculosis		induces ER stress in macrophages	unknown	66
Helicobacter pylori	VacA	induces the UPR	PERK activation	67,68
Listeria monocytogenes	Listeriolysin O (LLO)		intracellular Ca^{2+} imbalance?	70
Brucella melitensis	TcpB	induces the UPR	unknown	69
Brucella abortus	VceC	induces the UPR	BiP binding	62
	BspC, G, H, I, K	induces the UPR	unknown	60
Simkanianegevensis		inhibits the UPR	unknown	40
Escherichia coli (STEC)	SubtilaseSubAB	activates IRE1, ATF6, PERK	degradesBiP	75–77
	Shiga toxin 1 (Stx1)	induces the UPR	Ca^{2+} efflux from ER	103
Vibrio cholerae	Cholera Toxin (CT)	activates IRE1	IRE1 binding	2
various	Pore Forming Toxins (PFT)	induces the UPR	p38 MAPK pathway activation	74