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### Authors

Tsai, April Y  
English, Bevin C  
Tsolis, Renée M

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## Hostile takeover: hijacking of endoplasmic reticulum function by T4SS and T3SS effectors creates a niche for intracellular pathogens

April Y. Tsai, Bevin C. English, and Renée M. Tsolis\*

University of California, Davis, Department of Medical Microbiology & Immunology, One Shields Avenue, Davis, CA 95616

### SUMMARY

After entering a cell, intracellular pathogens must evade destruction and generate a niche for intracellular replication. A strategy shared by multiple intracellular pathogens is the deployment of Type III secretion system (T3SS) and Type IV secretion system (T4SS)-injected proteins (effectors) that subvert cellular functions. A subset of these effectors targets activities of the host cell's endoplasmic reticulum (ER). Effectors are now appreciated to interfere with the ER in multiple ways, including capture of secretory vesicles, tethering of pathogen vacuoles to the ER, and manipulation of ER-based autophagy initiation and unfolded protein response. These strategies enable pathogens to generate a niche with access to cellular nutrients and to evade the host cell's defenses.

### INTRODUCTION

Multiple intracellular pathogens utilize T3SS and T4SS to target functions of the host cell's ER. While pathogens such as *Legionella pneumophila* and *Brucella abortus* have long been known to replicate in association with the ER (1, 2), the connection of vacuoles containing other intracellular pathogens, such as *Coxiella burnetii* (3, 4), *Anaplasma* spp. (5, 6), and *Chlamydia trachomatis* and its relatives (7, 8) with the ER has been recognized relatively recently. However, manipulation of ER function is not limited to pathogens that replicate within a vacuole, as cytosolic pathogens such as *Orientia tsutsugamushi* (9, 10) and *Rickettsia rickettsii* (11) also target ER-based functions via secreted effectors to promote their intracellular growth.

Recent progress in large-scale analyses of secreted proteins and in genetic analysis of previously intractable intracellular bacteria such as *C. trachomatis*, *C. burnetii* and *Rickettsia* spp. has led to an explosion in identification of new T3SS and T4SS effectors, and for some of these effectors, exciting recent advances have revealed how their interactions with host components contribute to the intracellular replication cycle of these organisms. This review will focus on recent progress in understanding how interactions with the ER mediated by secreted effectors (primarily of T4SS and T3SS) promote infection by intracellular bacteria.

\*Corresponding author: rmtsolis@ucdavis.edu.

## THE ER: A BIOSYNTHESIS AND SIGNALING HUB OF THE CELL

The ER performs multiple functions that are critical to cellular homeostasis. Approximately one-third of the mammalian cell's proteome is targeted to the ER, and accordingly, its best-characterized role is that of the "factory" for correct folding of proteins that ultimately function in the plasma membrane, the extracellular space, or in secretory compartments such as the ER itself, the Golgi, secretory vesicles and lysosomes. Within the ER lumen, protein folding is assisted by ER-resident chaperones, such as the Hsp70 chaperone BiP, which binds hydrophobic protein regions, thereby preventing their aggregation (reviewed in (12)). The majority of secretory proteins are further modified by addition of glycans to asparagine residues, referred to as N-linked glycosylation. This modification increases the solubility and stability of hydrophobic proteins and promotes their cellular targeting and function (reviewed in (13)). As protein folding proceeds, resident ER proteins and chaperones also perform quality control to ensure that misfolded or aggregated proteins do not accumulate, as they can disrupt ER function. If a protein is terminally misfolded and cannot be refolded to a functional conformation, it is targeted to the ER-associated degradation (ERAD) pathway, wherein the misfolded protein is extracted from the ER membrane to the cytosol while being tagged with polyubiquitin chains, resulting in proteosomal degradation (reviewed in (14)).

In addition to its role in protein folding, the ER is site of lipid biosynthesis and central regulator of lipid levels throughout the cell (reviewed in (15)). The ER produces the main phospholipids composing cellular membranes, as well as less abundant membrane components. Enzymes that synthesize cholesterol are also located in the ER. After their synthesis, these lipids are distributed from the ER to their sites of function in the cell via the secretory pathway or via membrane contact sites with other organelles (see below). Further, under conditions of excess nutrition ER-localized enzymes synthesize triacylglycerides for energy storage within lipid droplets in the cell. Together, these ER-based functions are critical for maintaining cellular lipid homeostasis. As vacuolar pathogens replicate, their vacuole needs to expand, and thus an association with the ER could provide membrane lipids needed to enlarge the intracellular niche. Lipids produced by the ER might also provide biosynthetic material to intracellular pathogens for generation of membrane lipids or for energy (16).

Within the structure of the ER, specialized membrane domains are organized to carry out specific functions. Specific subdomains of the ER give rise to peroxisomes, organelles that sequester enzymes for  $\beta$ -oxidation of very long chain fatty acids as well as for metabolism of cholesterol, bile acids, and polyamines (17, 18). Another set of specialized ER domains are the membrane contact sites (MCS) that form between ER and other organelles in the cell, including mitochondria, Golgi apparatus, plasma membrane, endosomes and peroxisomes (reviewed in (19)). These are sites where organelles are tethered to each other via interactions between proteins in apposing membranes. The membrane contact sites between ER and mitochondria, for example, are extensive, and play essential roles in mitochondrial division (20) and calcium signaling between ER and mitochondria (21, 22). The ER proteins VAPA and VAPB tether multiple organelles in the cell to the ER via MCS, including the Golgi, endosomes and plasma membrane (19). Of particular interest for thinking about how

pathogens could associate with the ER after uptake, it is now appreciated that endosomes associate with the ER, and these contacts become more extensive as endosomes mature—in fact, endosomes remain tightly associated with the ER throughout their trafficking (23), suggesting a potential point of contact between pathogen-containing endosomes and the ER that might be exploited by pathogens.

In response to a stimulus such as amino acid starvation, yet another specialized ER domain known as the omegasome forms, providing one of the pathways to initiate autophagy, a process in which cellular components are recycled to provide nutrition to the cell (reviewed in (24)). The omegasome contains the protein DFCP1 and is enriched in phosphatidylinositol-3-phosphate, which is thought to increase the membrane curvature to initiate formation of the phagophore, the double membrane that is characteristic of autophagosomes (25).

## BACTERIAL STRATEGIES FOR CO-OPTING ER FUNCTION

Recent work has identified how the ER functions outlined above can be subverted by intracellular bacterial pathogens to generate a replicative niche, gain nutrients for growth, or spread from cell to cell. While *Brucella abortus* was recently shown to replicate with the ER lumen (26), other pathogens including *Legionella pneumophila*, *Chlamydia* spp., *Simkania negevensis*, *Anaplasma* spp., and *C. burnetii*, reside in a vacuole that during some part of their replicative cycle is tethered to the ER via membrane contact sites between the pathogen-containing vacuole and the ER (2, 4, 6-8). Yet another group of pathogens, exemplified by *R. rickettsii* and *Orientia tsutsugamushi*, reside in the host cell's cytosol and secrete effectors that target ER functions (11, 27). The following section will review recent advances in our understanding of how pathogen effectors interact with the ER.

### Subversion of Vesicular Trafficking Between ER and Golgi Apparatus

*Brucella abortus*, a zoonotic pathogen causing abortion in ruminants and febrile infections in humans, utilizes its T4SS to replicate intracellularly in multiple cell types, with the macrophage being the best studied (reviewed in (28)). After uptake by macrophages, *B. abortus* is able to avoid degradation in lysosomes (reviewed in (29)) and replicates within the ER (26). To establish this replicative niche, *B. abortus* utilizes its T4SS to interact with ER exit sites, where ER-to-Golgi transport is initiated, in a manner that is dependent on the small GTPase Sar1 (30), though the effectors mediating the association with ER exit sites have not yet been identified (Fig. 1). *B. abortus* also requires Golgi-to-ER transport for the maintenance of its replicative niche, as the small GTPase Rab2 contributes to intracellular replication of *B. abortus* (31). To date, approximately 15 T4SS effectors have been shown to be translocated into infected host cells (28). Recently, the T4SS effector BspB (Table 1) was found to alter secretory trafficking from both ER to Golgi and from the Golgi to the ERGIC (ER-to-Golgi Intermediate Compartment) to the ER by interacting with the Conserved Oligomeric Golgi (COG) complex (24). This interaction between BspB and the COG complex diverts Golgi-derived vesicles to *Brucella's* replicative compartment, thereby promoting its intracellular replication—possibly by providing membrane for expansion of the bacterial niche (Fig. 1). In addition to BspB, several *B. abortus* effectors including BspA,

BspD, BspK and VceC accumulate in the ER after ectopic expression (32–35), suggesting that they may perturb the early secretory pathway, but how these effectors function remains to be determined.

*Legionella pneumophila*, which naturally infects amoebae but causes opportunistic respiratory infections in humans, also uses its T4SS (called Dot/Icm) to target trafficking between the Golgi and the ER (reviewed in (36)). Of the over 300 Dot/Icm effectors identified to date, a subset targets the function of the early secretory pathway. RalF, the first *L. pneumophila* T4SS effector to be identified, acts as a GEF for ARF1, a small GTPase that regulates secretory membrane transport, primarily between the Golgi and ER (37). Several effectors target Rab1, the small, membrane-associated GTPase that regulates ER-to-Golgi vesicular transport. Rab1 cycles between GDP-bound (inactive) and GTP-bound (active) forms, with the assistance of multiple cellular factors (Fig. 1 and Table 1). Guanine nucleotide exchange factors (GEFs) activate Rab1 by converting GDP-Rab1 into GTP-Rab1. GTP-Rab1 then interacts with its target proteins in the membrane transport pathway to promote tethering and fusion of membrane vesicles (reviewed in (38)). To inactivate GTP-Rab1, GTPase activating proteins (GAPs) stimulate the GTPase activity of Rab1 to convert it to inactive GDP-Rab1. The interaction of GDP-Rab1 with membranes is regulated by GDP dissociation inhibitor (Rab-GDI), which extracts it from membranes, and by a GDI displacement factor (GDF), which targets Rab1-GDP to membranes to re-start the Rab cycle. Rab1 is recruited to the *Legionella*-containing vacuole (LCV) via the activity of the Dot/Icm effector SidM (also known as DrrA; (39)). Biochemical analysis of SidM has revealed multiple activities for modulating Rab1 activity (reviewed by (36, 40)). A C-terminal phosphatidylinositol-4-phosphate (PI4P) binding domain of SidM mediates its association with the LCV after its secretion by the T4SS (41). The central domain of SidM has GDF/GEF activity for Rab1, which displaces Rab-GDI from Rab1-GDP and mediates GDP exchange for GTP, leading to its association with the membrane of the LCV. After recruiting Rab1 to the LCV, SidM uses its N-terminal domain, which contains adenylyltransferase activity, to covalently modify Rab1 by AMPylation (adenylylation) of a tyrosine residue. This modification of Rab1 prevents its interaction with GAPs, so as a result, Rab1 remains in its GTP-bound form and becomes constitutively active (42). Modification and recruitment of Rab1 by SidM is assisted by a second Dot/Icm effector, LidA (39, 43), which interacts with GTP-bound Rab1 (and with other Rab-GTP complexes as well (44, 45)). This manipulation of Rab1 activity enables *L. pneumophila* to recruit ER-derived vesicles, thereby remodeling its phagosome into a compartment supporting its replication (46).

Over the time of cellular infection by *L. pneumophila*, additional effectors are translocated that act in an antagonistic manner to SidM and LidA on Rab1 activity. SidD acts to de-AMPylation Rab1 (47), which restores its GTPase activity. Subsequently, LepB promotes hydrolysis of GTP by Rab1 (48), thereby enabling its extraction from the LCV by host Rab-GDI proteins. The time course of effector secretion and Rab1 modulation by *L. pneumophila* suggests that early during infection, recruitment and activation of Rab1 is beneficial for replication. However, prolonged activation of Rab1 may elicit cellular responses that are detrimental to intracellular infection, since ectopic expression of SidM/DrrA is cytotoxic to cells (42).

An additional covalent modification of Rab1, phosphocholination, is mediated by AnkX (49). Interestingly, while AMPylation targets a conserved tyrosine residue in Rab1, AnkX targets the adjacent serine residue. AnkX contains both ankyrin repeat domains and a FIC (filamentation induced by cyclic AMP) domain, which utilizes CDP-choline as a substrate for phosphocholination of Rab1 (50). It appears that Rab1 can only be either AMPylated or phosphocholinated at once, as only one or the other modification was identified per Rab1 molecule (50). Phosphocholination of Rab1 appears to promote its activity in a manner similar to AMPylation. Similarly to AMPylation, AnkX-mediated phosphocholination can be reversed by a second effector, Lem3 (51, 52). It was recently found that an endogenous host protein, TGF $\beta$ -activated kinase (TAK1) regulates Rab1 by phosphorylation at the same site modified by AnkX and SidM, suggesting that these T4SS effectors mimic the host's own regulatory mechanism to co-opt Rab1 function (53).

One puzzling observation is that despite the multiple effectors that modulate Rab1 activity during *Legionella* infection, Rab1 itself appears to be dispensable for intracellular replication. A possible explanation for this finding is that a subset of effectors, such as SidM, AnkX and LidA, appear to target multiple GTPases (44, 45, 50), and that these additional activities may act in parallel with perturbation of Rab1 function to promote the intracellular life cycle of *L. pneumophila*.

### Tethering of Pathogen-Containing Vacuoles to the ER

Several intracellular pathogens, including *L. pneumophila*, *C. burnetii* (3, 4), *Anaplasma* spp. (5, 6), and *Chlamydia* spp. replicate in vacuoles that are closely associated to the ER but do not appear to fuse with it. This lifestyle is shared by *Simkania negevensis*, an organism related phylogenetically to *Chlamydia*, that naturally infects amoebae, and similar to *L. pneumophila*, causes opportunistic respiratory tract infection (7, 8). While the effectors mediating association with *C. burnetii*, *Anaplasma* spp and *S. negevensis* with the ER remain to be identified, recent work has identified T3SS and T4SS effectors that promote association of vacuoles containing *C. trachomatis* and *L. pneumophila* with the ER (Fig. 2).

*Chlamydia* spp. are obligately intracellular pathogens that cause genital tract and ocular (*C. trachomatis*) or respiratory tract (*C. pneumoniae* and *C. psittaci*) infections. Both pathogens replicate within a vacuole termed an inclusion that has membrane contact sites with the ER. In *C. trachomatis*, the T3SS substrate IncD localizes to the inclusion membrane and mediates contact with the ER at membrane contact sites that also contain VAPA/B, the lipid transfer protein CERT, and the ER calcium sensor STIM1 (7, 54). A second T3SS effector, IncV, interacts with VAPA/B at the membrane contact sites between the inclusion and the ER (55). A *C. trachomatis incV* mutant exhibited decreased association of its inclusion with the ER but no overall intracellular growth defect, suggesting both the importance of IncV in ER tethering and the involvement of additional effectors in this process. Association of the chlamydial inclusion with the ER, especially with CERT, may promote acquisition of lipids to promote replication of *Chlamydia* either for nutrition or for expansion of the inclusion membrane during bacterial replication.

*C. burnetii* is a zoonotic pathogen that causes Q fever, that can manifest with both acute and chronic pathology (56). It utilizes a T4SS related to the *Legionella* Dot/Icm apparatus to

promote its intracellular replication (57, 58). Over 100 *C. burnetii* Dot/Icm substrates have been identified to date, but only a few have been characterized functionally (56). The vacuole containing *C. burnetii*, termed the parasitophorous vacuole, is decorated with calnexin and is tethered to the ER the ER via membrane contacts that contain the host sterol-binding protein ORP1L, a protein that interacts with VAPA/B at ER membrane contact sites (4). While the effectors mediating this tethering remain to be identified, multiple T4SS effectors, including Cbu0372, Cbu1576 and ElpA localize on ectopic expression to the ER (59, 60), and Cbu0635 interferes with the secretory pathway (58) suggesting that these and/or other T4SS effectors may play a role in interactions with the ER.

Recent evidence suggests tethering of vacuoles containing *Legionella* to the ER during the early stage of infection (Fig 2). The Dot/Icm effector Ceg9 interacts with the ER protein Reticulon-4 (Rtn4) shortly after uptake of bacteria, suggesting that recruitment of ER helps to develop the replicative niche for *L. pneumophila* (61). In the host cell, Rtn4 helps generate the tubular morphology that is characteristic of the peripheral ER (62) and participates in the formation of ER-plasma membrane MCS that function in cellular Ca<sup>2+</sup> homeostasis (63). Like Rab1, Rtn4 is targeted by multiple Dot/Icm effectors, including the SdeA-C proteins, which modulate Rtn4 function via ubiquitination (64). Intriguingly, the Sde proteins perform a sequential set of reactions to transfer ubiquitin to Rtn4: ADP-ribosyl transfer to ubiquitin, followed by a nucleotidase/phosphohydrolase reaction that removes AMP and transfers phosphoribosylated ubiquitin to Rtn4 (64). The early targeting of Rtn4 after *L. pneumophila* entry to the cell and the localization of Rtn4 to plasma membrane-ER MCS raise the possibility that *L. pneumophila* could co-opt these MCS early during cellular infection to associate with the ER. Another Dot/Icm substrate involved in tethering the LCV to the ER is SidC, which is anchored to the cytosolic face of the LCV via binding of phosphatidylinositol-4 phosphate (65-67). Recruitment of ER proteins to the LCV by SidC requires a ubiquitin ligase activity in its N terminus (68). Taken together, these findings suggest that pathogens use multiple strategies to tether their replicative vacuoles to the ER.

### Subversion of Autophagy Initiation at the ER

The *L. pneumophila* T4SS effector protein RavZ is secreted from the LCV and targets to omegasomes via its ability to interact with the lipid phosphatidylinositol-3-phosphate (PI(3)P) that is enriched at these sites (69). There, the cysteine protease activity of RavZ irreversibly deconjugates lipids from ATG8 proteins (LC3-II) in the early-stage autophagosomal structures. As a result, the biogenesis of autophagosomes at the ER is inhibited (70). Since a *ravZ* mutant does not have a replication defect (70), it is unknown whether this activity promotes intracellular replication of *L. pneumophila*, however RavZ may act in concert with other effectors such as the inhibitor of sphingolipid biosynthesis LpSpl (71) to modulate autophagy. In contrast to *L. pneumophila*, *Anaplasma phagocytophilum*, which replicates within neutrophils, activates autophagy via a T4SS effector, Ats-1, to promote its replication (5). Ats-1 has an N-terminal domain that nucleates autophagosomes by interacting with Beclin 1, a protein crucial to initiation of autophagy, to recruit the ER-localized autophagy initiation proteins ATG14L and DFCP1 to the *A. phagocytophilum* inclusion (5). This subversion of autophagy initiation recruits

autophagosomes to the inclusion, effectively delivering nutrients for intracellular replication of *A. phagocytophilum* (72).

### Effector Modulation of the ER Unfolded Protein Response (UPR)

The UPR is a response to perturbation of ER function (broadly termed ER stress) that is initially cytoprotective and promotes return to homeostasis, but can lead to apoptosis in the case of unresolved stress. The cellular response to ER stress is transmitted via three membrane sensors, IRE1 $\alpha$  (inositol-requiring enzyme 1), ATF6 (activating transcription factor 6) and PERK (protein kinase RNA-like ER kinase). This response is linked to innate immunity via signaling through cytosolic pathways (reviewed in (73, 74)). Secreted effectors have been identified that both induce and inhibit the UPR during intracellular infection. The *B. abortus* T4SS effector VceC localizes on ectopic expression to the ER, and during infection it activates IRE1 $\alpha$ , initiating a proinflammatory arm of the UPR (32, 34) that activates NF- $\kappa$ B. This response could be beneficial to *B. abortus* in the bovine placenta, as placental inflammation in this context triggers abortion, driving transmission of the pathogen in its natural reservoir (32, 34). A second T4SS effector, BtpA (also called TcpB or Btp1) (75) triggers all three branches of the UPR during *Brucella melitensis* infection (76), but rather than localizing to the ER, BtpA binds microtubules (77, 78). It is not known how microtubule stabilization by BtpA links to UPR induction, but potential mechanisms could include altering interaction of integral ER membrane proteins with microtubules or effects on microtubule-dependent vesicular transport in the secretory pathway (79).

Like *B. abortus*, *O. tsutsugamushi*, the obligate intracellular agent of scrub typhus, activates the UPR. (9, 27). Two T1SS-secreted effectors have been implicated (Table 1): Ank4, an ankyrin repeat protein, interacts at the cytosolic face of the ER with Bat3, a host cytosolic chaperone involved in ERAD (80), to inhibit UPR-induced ERAD during the early (non-replicative) phase of *O. tsutsugamushi* infection. Later, Ank4 expression is downregulated, which releases repression of ERAD, making amino acids available for intracellular replication of *O. tsutsugamushi*, which provides an important source of nutrition, since this bacterium is auxotrophic for several amino acids (81). Ectopically expressed Ank9 binds the Golgi protein COPB2 involved in Golgi-to-ER vesicular trafficking, and Ank9 also traffics from Golgi to the ER, where it disrupts organelle morphology and induces the UPR. Ectopic Ank9 expression phenocopies disruption of Golgi and ER, as well as inhibition of protein secretion, observed in cells infected with *O. tsutsugamushi* (27).

*L. pneumophila* activates the UPR at the transcriptional level (82) but suppresses the downstream translation of UPR target transcripts by translocating five T4SS effectors (Lgt 1-3, SidI and SidL) that inhibit translation elongation. The *L. pneumophila* effectors function by glycosylation of a conserved serine residue in host elongation factor 1A (eIF1A; (83)). Translation inhibition effectively reduces the basal load of protein entering the ER for protein folding, which is a physiologic activator of IRE1 $\alpha$  (82, 84), thus the outcome of this interaction is to inhibit the IRE1 $\alpha$  pathway. The ability to block IRE1 $\alpha$  signaling is shared by *S. negevensis*, which encodes both T3SS and T4SS in its genome, however the effectors that mediate this activity remain to be discovered (8). Blockade of the IRE1 $\alpha$  pathway may be beneficial in the context of bacterial infection either to reduce innate immune signaling



downstream of this pathway or to block induction of apoptosis in response to uncontrolled ER stress. Interestingly, blockade of the UPR is a strategy shared by viral pathogens, which via their subversion of the ER for production of virions, trigger ER stress (85).

## CONCLUSIONS AND PERSPECTIVE

The biosynthetic capacity of the ER and its extensive network of contacts with other cellular organelles make it a logical target for exploitation by T3SS and T4SS of different intracellular pathogens. Pathogens such as *L. pneumophila* and *C. burnetii* have dedicated a substantial part of their genome coding capacity to secreted effectors that modulate their host cells, which highlights the importance of these interactions to their biology (56, 86, 87). Recent progress in understanding the biology of T3SS and T4SS effectors has revealed novel mechanisms utilized by intracellular bacteria to co-opt multiple functions of the ER, including protein secretion, lipid biosynthesis, membrane tethering and autophagy initiation, to promote their replication. While our understanding of how individual effectors modulate ER function is growing, for the majority of effectors, the molecular mechanisms of action remain unknown.

One of the challenges to identifying effector functions and understanding their roles in the context of infection has been the redundancy of effector function. However, elegant approaches have been employed in *C. trachomatis* and *Mycobacterium tuberculosis* to generate interaction networks (88, 89) for secreted effectors, that together with newly-developed methodologies for genetic manipulation of the obligate intracellular pathogens, will facilitate functional and mechanistic studies of effector proteins and uncover new strategies by which they manipulate ER biology.

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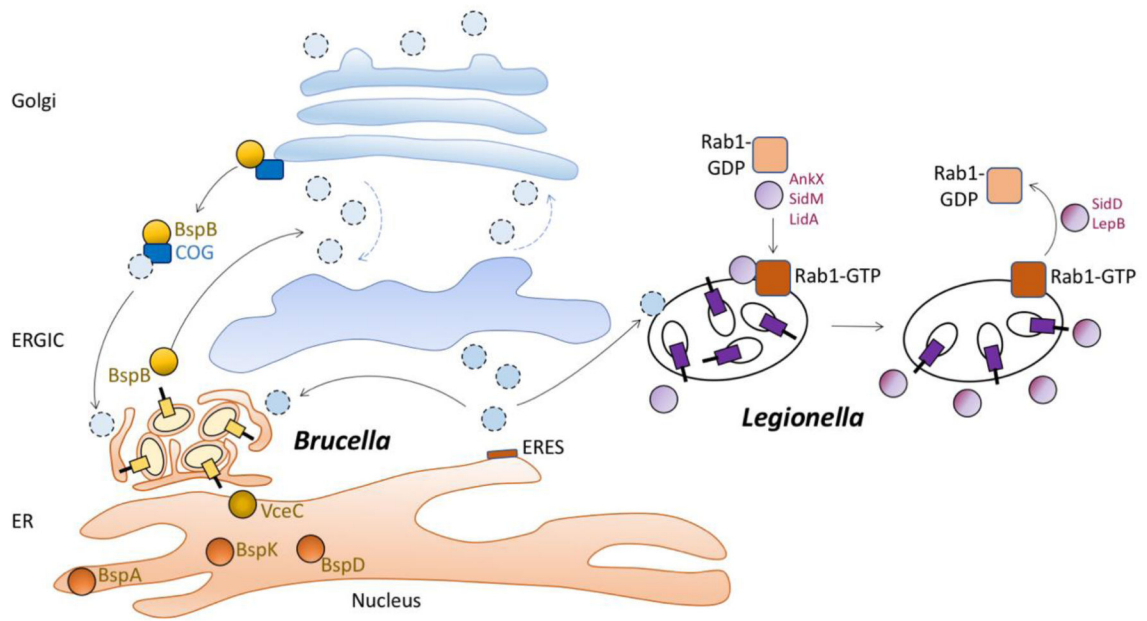
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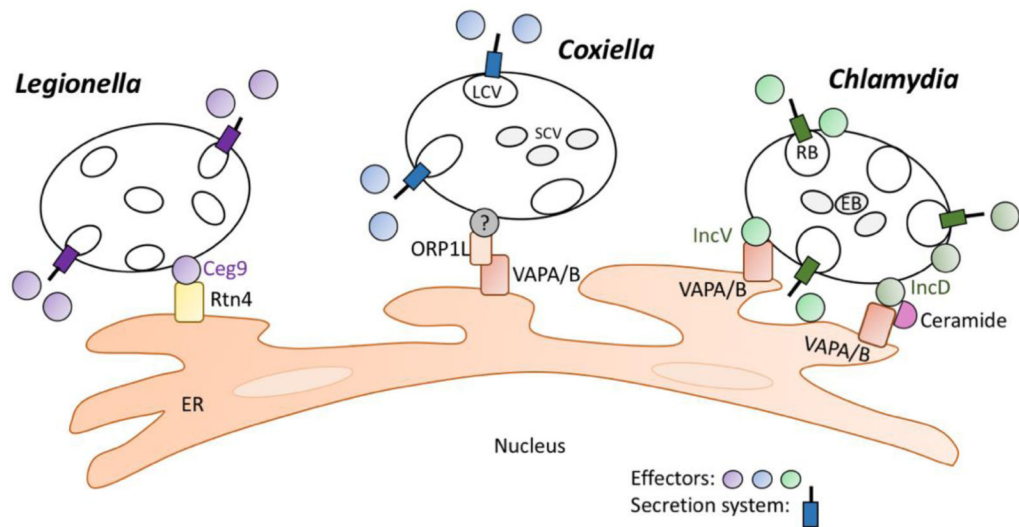
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**Figure 1:** Hijacking of vesicular traffic between ER and Golgi by T4SS effectors of *B. abortus* (left) and *L. pneumophila* (right). It has been proposed that like *B. abortus*, *L. pneumophila* also intercepts Golgi-ER traffic (109). Abbreviations: ERGIC, ER-to-Golgi Intermediate Compartment; ERES; ER exit site; COG, conserved oligomeric Golgi complex; GTP, Guanosine triphosphate; GDP, Guanosine diphosphate





**Figure 2:**

Role of pathogen effectors in tethering of pathogen-containing vacuoles to the ER.

Abbreviations: Rtn4, reticulon-4; LCV, *Coxiella* large cell variant; SCV, *Coxiella* small cell variant; RB, *Chlamydia* reticulate body; EB, *Chlamydia* elementary body

Table 1:

## Secreted pathogen effectors that localize to the ER or modulate its function

Pathogen (secretion system)	Effector	Activity	References
<i>Brucella abortus</i> (VirB T4ASS)	BspB	Impairs ER to Golgi secretory trafficking; Interacts with the COG complex in the Golgi and redirects membrane vesicles from Golgi to <i>Brucella</i> vacuole	(90)
	VceC	Localizes to ER (ectopic expression); Induces ER stress; Interacts with BiP/GRP70	(32-34)
	BtpA (TcbB/Btp1)	Induces ER stress; Binds microtubules; Inhibits TLR signaling	(76) (77, 78) (75)
	BspA	Unknown; localizes to ER on ectopic expression	(35)
	BspD	Unknown; localizes to ER on ectopic expression	(35)
	BspK	Unknown; localizes to ER on ectopic expression	(35)
<i>Legionella pneumophila</i> (Dot/Icm T4BSS)	Lgt1	Inhibits the IRE1 pathway of the UPR; Inhibits translation elongation by glucosylation of eukaryotic elongation factor 1A (eEF1A)	(82-84)
	Lgt2	Inhibits the IRE1 pathway of the UPR; Inhibits translation elongation by glucosylation of eukaryotic elongation factor 1A (eEF1A) Localizes to the cytoplasmic face of the LCV; regulates	(82, 84, 91)
	SidE	ER tubule rearrangement and recruitment of ER markers to the LCV via modulating ubiquitination	(64, 92-94)
	SidC	Ubiquitin ligase and PI4P binding activity; Promotes the association of LCVs with the ER by recruiting ER vesicles.	(66, 68, 95)
	SdeA, B, C	Promote ER reorganization by progressive ADP-ribosylation of ubiquitin and transfer of phosphoribosyl moiety to Reticulon 4 (Rtn4)	(64, 93, 94, 96)
	Ceg9	Tethers the <i>Legionella</i> -containing vacuole (LCV) to the ER via association with Rtn4	(61)
	SidM/DrrA	Recruits Rab1 to LCV; Acts as a guanosine nucleotide exchange factor (GEF) to recruit vacuoles to the LCV and as a GDI-displacement factor (GDF) for Rab1; AMPylates Rab1	(39, 41, 42, 47, 50, 97-99)
	LidA	Interacts with GTP-Rab1 to maintain it in the active conformation	(43, 45, 100)
	SidD	Catalyzes AMP release from Rab1	(47)
	AnkX	Transfers phosphocholine to Rab1	(50)
	RalF	Acts as a GEF to activate ARF	(37)
	Lem3	Reverses activity of AnkX by removing phosphocholine from Rab1	(52)
	SetA	Glycosylates Rab1	(101, 102)
	LepB	Inactivates Rab1 via RabGAP activity; Manipulates phosphoinositide composition of the <i>Legionella</i> -containing vacuole via phosphatidylinositol 4-kinase activity	(48, 103, 104)
	Lpg1137	Cleaves Syntaxin 17 at the mitochondria-associated ER membrane and blocks autophagy	(105)
RavZ	Delipidates Atg8 (LC3-II) at the phagophore to inhibit autophagosome formation	(70)	
LpSpl	Sphingosine-1-phosphate lyase disrupts host sphingolipid biosynthesis and inhibits autophagy during infection	(71)	
<i>Chlamydia trachomatis</i> (T3SS)	CT229	<i>C. pneumoniae</i> homolog Cpn0585 recruits Rab1 from the ER to the inclusion membrane (effector that recruits Rab1 to <i>C. trachomatis</i> inclusion has yet to be identified).	(106)
	IncD	Mediates contact with the ER at membrane contact sites via binding to ceramide transfer protein CERT	(54, 107)
	IncV	Tethers the <i>C. trachomatis</i> inclusion to the ER via interactions with VAPs	(55)

Pathogen (secretion system)	Effector	Activity	References
	MrcA	Interacts with the Ca <sup>2+</sup> channel inositol-1,4,5-trisphosphate receptor, type 3 (ITPR3) to promote release of bacteria from infected cells	(108)
<i>Coxiella burnetii</i> (Dot/Icm T4BSS)	ElpA	Localizes to the ER on ectopic expression and blocks secretory traffic.	(59)
<i>Anaplasma</i> spp. (VirB T4ASS)	Ats-1	Nucleates autophagosomes by interacting with Beclin and recruitment of DFPC1 and ATG14L to the pathogen-containing vacuole	(5, 72)
<i>Orientia tsutsugamushi</i> (T1SS)	Ank4	Interacts with eukaryotic chaperone Bat3 to transiently impede ER-associated protein degradation.	(9)
	Ank9	Destabilizes the ER and Golgi by binding COPB2 and induces ATF4-dependent UPR	(27)
<i>Rickettsia rickettsii</i> (Rvh T4ASS)	RARP-2	Forms membranous structures in association with the ER; contributes to lysis of infected host cells.	(11)