# The *Legionella pneumophila* replication vacuole: making a cosy niche inside host cells

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Abstract | The pathogenesis of *Legionella pneumophila* is derived from its growth within lung macrophages after aerosols are inhaled from contaminated water sources. Interest in this bacterium stems from its ability to manipulate host cell vesicular-trafficking pathways and establish a membrane-bound replication vacuole, making it a model for intravacuolar pathogens. Establishment of the replication compartment requires a specialized translocation system that transports a large cadre of protein substrates across the vacuolar membrane. These substrates regulate vesicle traffic and survival pathways in the host cell. This Review focuses on the strategies that *L. pneumophila* uses to establish intracellular growth and evaluates why this microorganism has accumulated an unprecedented number of translocated substrates that are targeted at host cells.

Functional redundancy
Two proteins that perform a similar or related function; for example, when the activity of one protein can compensate for the absence of the other.

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Many bacterial and eukaryotic parasites trick host cells into providing comfortable living arrangements for their descendents. Some of these microorganisms have similar requirements to viruses, as they cannot grow in extracellular or environmental niches and must instead establish an intracellular replication cycle. Other intracellular microorganisms can replicate either inside or outside host cells. The intracellular lifestyle of these microorganisms allows them to gain a competitive advantage relative to other microorganisms or to colonize a host. Life inside cells could either enable evasion of killing mechanisms that are wielded by predatory cells in the environment, such as amoebae, or provide a niche to evade host humoral and cellular immune responses.

Following the uptake of microorganisms into a host cell membrane-bound compartment (called a vacuole in this Review), intracellular growth occurs. This growth involves replication either within the vacuole or in the host cell cytoplasm after destruction of the vacuole. For microorganisms that replicate in a vacuole, three important problems must be tackled. First, membrane-bound compartments newly formed from the host cell surface normally enter the antimicrobial lysosomal network, which is an inhospitable environment. Second, the microorganism must acquire sustenance through the vacuolar membrane. Third, microorganisms must deal with space limitations after they have begun to divide in this compartment. Intravacuolar pathogens, such as *Legionella pneumophila*, overcome these problems by establishing an intimate association with a particular organelle in the host cell secretory system and hijacking membrane traffic from this site to the pathogen-containing vacuole (PCV). The resulting PCV is camouflaged and provided with a ready supply of new membrane to satisfy the needs of a growing population.

In this Review, we describe the membrane traffic that leads to formation of the *L. pneumophila* PCV and replication of this microorganism within host cells. Important bacterial and host cell proteins that are necessary for intracellular replication will be analysed, as well as confounding results which indicate that functional redundancy exists among the proteins associated with formation of the PCV. We also present a model that attempts to explain the evolutionary basis for this redundancy. Finally, we discuss events that interfere with replication of *L. pneumophila* in host cells and the strategies used by this microorganism to overcome these blocks to replication.

## *L. pneumophila* — an intravacuolar pathogen

*L. pneumophila*, the causative agent of Legionnaires' disease, which leads to pneumonia, is an intravacuolar pathogen of environmental protozoa[1]. Pneumonia is initiated in humans after they inhale contaminated water supplies found in poorly designed air-conditioning units or sludge-filled plumbing[2], and infection might also result from infection by amoebae laden with bacteria[3].

Phagolysosome
A membrane-bound organelle that is formed through the fusion of a phagosome with a lysosome.

The primary site of replication of this Gram-negative bacterium is the alveolar macrophage, in which it grows within a membrane-bound compartment that is morphologically indistinguishable from that found during growth within amoebae[4,5].

The intravacuolar lifestyle of *L. pneumophila*[6–8] is summarized in FIG. 1. The bacteria are found in a vacuole that resists fusion with lysosomes, as shown by a number of different assays[6]. In support of the idea that trafficking of the *Legionella*-containing vacuole (LCV) is distinct from that of non-pathogens, the LCV is more resistant to acidification than compartments that contain *Escherichia coli*, indicating that maturation of the LCV into a phagolysosome is impeded[8]. Additionally, a series of alternative docking events seems to take place, including recruitment of mitochondria followed by association of ribosome-studded membranes (later shown to be endoplasmic reticulum (ER)) with the vacuolar membrane[7,9,10]. When either intact cells or isolated LCVs are analysed, ER-associated proteins are found localized near the vacuole shortly after uptake of *L. pneumophila*[11,12]. These ER-derived proteins include Sec22b, a member of the SNARE family of membrane fusion proteins, and the small GTPase Rab1, a regulator of traffic from the ER to the Golgi[11,12]. Although ER-derived material might be sequestered more slowly in amoebae than in macrophages[13], it is clear that the LCV assumes an ER character before rough ER surrounds the compartment[7] (FIGS 1,2).

The association of ER material with the LCV indicates that after entry into host cells *L. pneumophila* hijacks membrane material that is normally destined for fusion with downstream compartments, such as the Golgi apparatus[14]. In support of this model, interference with the function of Arf1 (ADP-ribosylation factor 1), a small GTPase that controls a large number of functions in the host cell, including the assembly of COPI (coatomer protein complex I) coats, which form and maintain the integrity of vesicles that are exiting from sites in the early secretory system, disrupts formation of the LCV[14]. Although Arf1 is usually associated with the budding of vesicles from the Golgi, the defect caused by overproduction of dominant negative Arf1 is probably due to the blocking of vesicle maturation from the ER, as there is little evidence for movement of vesicles in a retrograde direction from the Golgi to the LCV. Furthermore, dominant interfering mutants of Sar1, a small GTPase that is involved in the formation of vesicles that exit from the ER, also disrupt formation of the replication vacuole[14] (FIG. 2).

There is evidence to indicate that vesicles which exit the ER fuse with, and deposit their luminal contents into, the LCV. Fusion between vesicles and membranous compartments in eukaryotic cells requires SNARE proteins on both membranes. The association with the LCV by the Sec22b SNARE protein, which is normally found on donor vesicles that are derived from the ER, indicates that at least some of the host cell fusion machinery is available to allow docking and fusion of these vesicles with the LCV. The fact that a fragment of membrin, a SNARE protein found on acceptor compartments that normally acts as a partner to Sec22b, interferes with the formation of replication vacuoles is consistent with fusion in ER-derived vesicles[12]. Furthermore, several hours after uptake of the bacterium into macrophages, soluble ER-derived proteins, such as glucose-6-phosphatase and protein disulphide isomerase, can be detected within the LCV by electron microscopy, which indicates that the soluble contents of the ER are delivered to the lumen of the LCV[15].

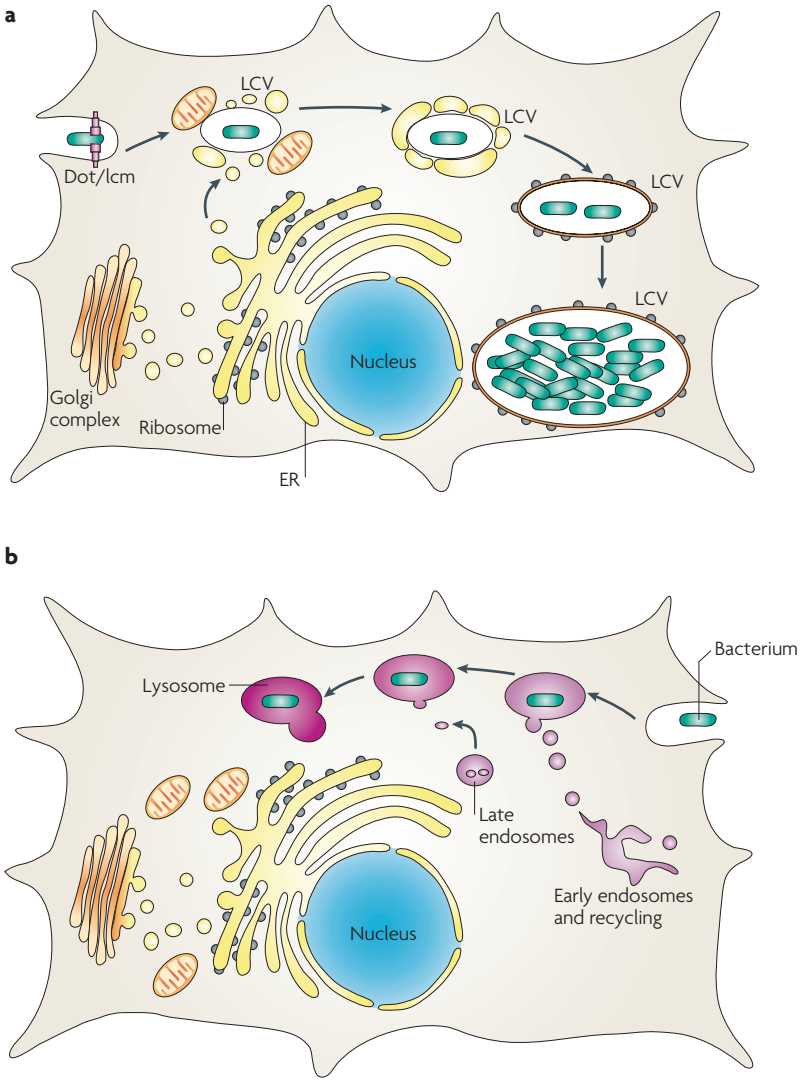


Figure 1 | ***Legionella pneumophila* modulates the trafficking of its vacuole to establish a replicative niche. a** | Formation of the replication vacuole. After uptake into target amoebae or macrophages, the *Legionella*-containing vacuole (LCV) evades transport to the lysosomal network and is sequestered in a compartment that is different from those observed for non-pathogens[6,7]. Within minutes of uptake, vesicles derived from the endoplasmic reticulum (ER; yellow compartments) and mitochondria appear in close proximity to the LCV surface. The identity of the ER-derived vesicles is based on the presence of proteins that are known to be associated with the early secretory apparatus. The vesicles that surround the LCV appear to be docked and extend out onto the surface, and eventually, the membranes that surround the bacterium become similar to rough ER in appearance and become studded with ribosomes. Within this ER-like compartment, the bacterium replicates to high numbers and eventually lyses the host cell. **b** | Default pathway of trafficking a non-pathogen. After bacterial uptake, the membrane-bound compartment acquires the character of early endosomes and late endosomes before entering the lysosomal network. Dot/Icm, defect in organelle trafficking/intracellular multiplication.

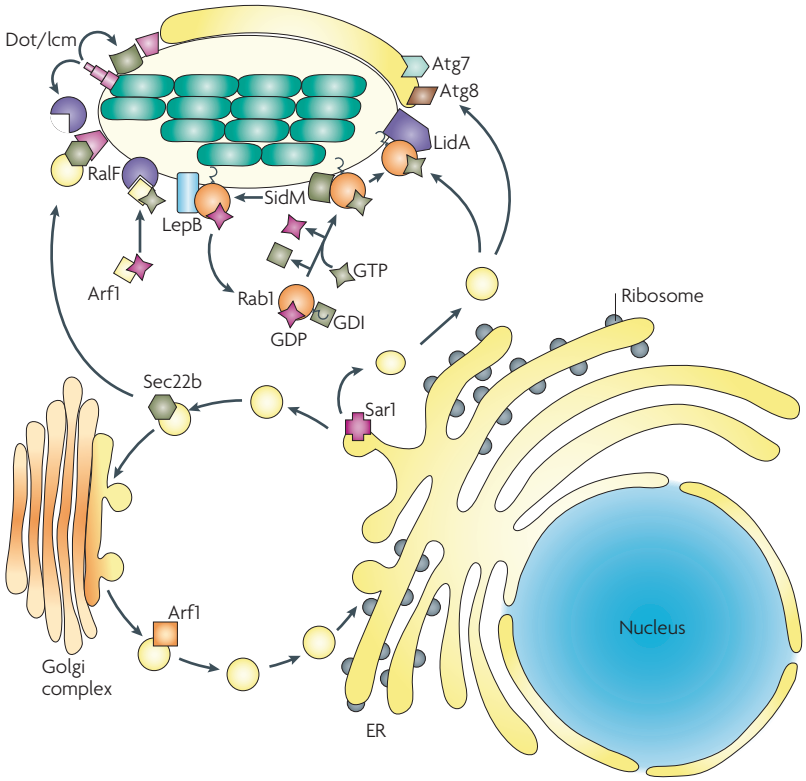


Figure 2 | **The *Legionella*-containing vacuole.** *Legionella pneumophila* proteins secreted via the Dot/Icm (defect in organelle trafficking/intracellular multiplication) translocation system associate with the *Legionella*-containing vacuole (LCV) and recruit host proteins that are involved in vesicle trafficking through the early secretory pathway. To simplify the components, the Dot/Icm apparatus is depicted as a tube that extends from the bacterial cytoplasm into the host cytosol, but there is no mechanistic support for this simplistic view. Sec22b, which is involved in the docking of endoplasmic reticulum (ER)-derived vesicles at the Golgi, is recruited to the LCV, although the mechanism of recruitment is unclear[12]. Rab1, another vesicle docking and fusion protein, is recruited to the LCV by the *L. pneumophila* protein SidM[76] (also known as DrrA[77]), which functions as both a Rab1 GDF (guanine nucleotide-dissociation inhibitor (GDI) dissociation factor[78,83]) and a Rab1 GEF (guanine nucleotide exchange factor[76,77]). LidA acts in conjunction with SidM to sequester activated Rab1 at the LCV membrane[76]. LepB is a RabGAP (Rab GTPase activating protein[78]), and may be involved in the dissociation of Rab1 from the vacuolar membrane. ADP-ribosylation factor 1 (Arf1), which is involved in vesicle budding and recycling at the Golgi, is recruited to the LCV by RalF, which functions as an Arf1 GEF[33]. Host membrane recruitment to the LCV might involve an autophagy process, as both the host autophagy proteins Atg7 and Atg8 also localize to the LCV[21].

COPI
(Coatomer protein complex I) A protein complex that coats the outer surface of vesicles that travel between the endoplasmic reticulum (ER)-to-Golgi intermediate compartment or are recycled from the Golgi complex to the ER.

Dominant negative
A mutation which leads to a phenotype that persists in the presence of the wild-type allele.

## Autophagy and intracellular replication

Although most studies have found that ER is associated with the LCV throughout intracellular replication, other membrane trafficking events may modulate the intracellular growth of *L. pneumophila*. One study found that the separation between the LCV and the endocytic network breaks down in mouse macrophages: replicating *L. pneumophila* was found in compartments that contain the late endosomal protein LAMP1 (lysosome-associated membrane protein 1)[16]. By contrast, another study argued that LAMP1 compartments are unlikely to exist during replication of *L. pneumophila* in other cell types[17]. In addition, in a cultured cell line, *L. pneumophila* seems to be released into the host cell cytoplasm, where the bacteria might undergo a few rounds of replication before host cell lysis[18].

Another possibility that has been raised regarding the biogenesis of the LCV is that the membranous material surrounding the LCV is derived from autophagy, which is initiated to clear *L. pneumophila* from the host cell[19]. During autophagy, cytoplasmic material is encapsulated by membranes that resemble the ER and packaged for eventual delivery to the lysosome, where the cargo is degraded[20]. The association of the LCV with markers of autophagy[21], such as the autophagy-related proteins Atg7 and Atg8, is consistent with the formation of a nascent compartment that is destined to be targeted for degradation. If this is the case, then autophagy must be arrested for the bacteria to maintain intracellular replication[22] (FIG. 2). However, mutants of the amoeba *Dictyostelium discoideum* that are defective for the formation of autophagous compartments show normal intracellular replication of *L. pneumophila*[23].

## The Dot/Icm machine

Efficient formation of the replication vacuole and successful intracellular growth of *L. pneumophila* requires most of the 27 *dot/icm* (defect in organelle trafficking/intracellular multiplication) genes[24–27] (see FIG. 3 for the presumed location of each component in the system; TABLE 1). Mutations in many of these genes lead to defective recruitment of ER-derived material to the LCV and rapid acquisition of late endosomal markers, such as LAMP1 (REFS 9,28). Most of the predicted protein products of these genes resemble components of conjugative DNA-transfer apparatuses (type IV secretion systems; T4SSs)[29]. Although there are multiple T4SSs in each of the four sequenced *L. pneumophila* strains[30,31], it was shown that bacteria can transfer DNA to other bacterial cells in a *dot/icm*-dependent manner, which indicates that the Dot/Icm machine transfers macromolecules to target cells[27,32]. Protein is probably the most important macromolecule that is transferred to host cells[33]. This was originally made clear by bioinformatic searches for proteins that show sequence similarity to eukaryotic proteins that manipulate ER-to-Golgi traffic. In this way, the RalF (recruitment of Arf1 to *Legionella* phagosome) protein was identified. RalF, which was shown to be translocated to macrophages in a Dot/Icm-dependent manner, has a Sec7 homology domain that allows the protein to activate Arf1 (REF. 34). Following this discovery, it became clear that the function of the Dot/Icm system was to deliver proteins across the target host cell membrane. These translocated substrates accumulate across the plasma membrane shortly after contact of the bacterium with the host cell[35], and are found on the outer surface of the LCV as well as associated vesicles[33]. It initially took a substantial period of time to identify only one single translocated protein, but the number of identified Dot/Icm substrates has since avalanched (discussed below).

Although our understanding of the functions of the Dot/Icm proteins is still poor, they can be separated into several classes, as described below (TABLE 1).

***Translocated substrate-associated proteins.*** The IcmS protein in complex with either IcmW or the virulence protein LvgA seems to coordinate the presentation

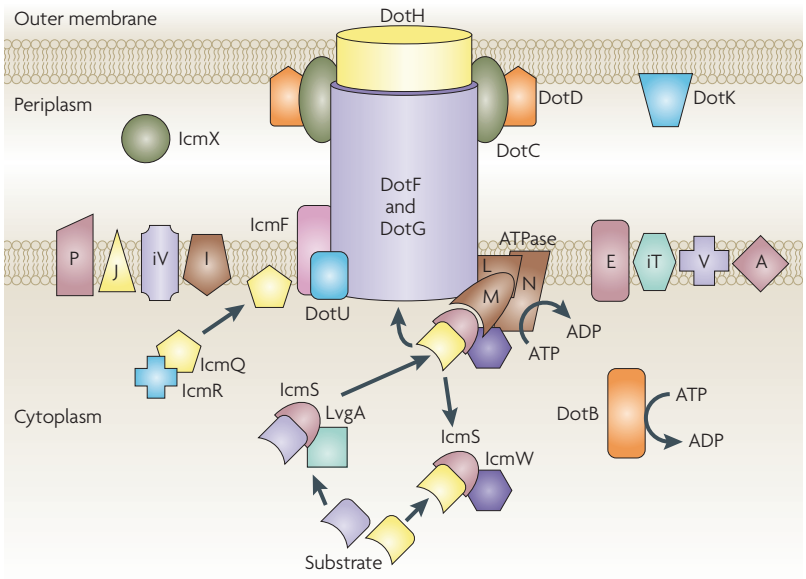


Figure 3 | **The Dot/Icm translocation apparatus.** The presumed locations and topological relationships of the various Dot/Icm (defect in organelle trafficking/intracellular multiplication) components in the *Legionella pneumophila* envelope are shown based on a study of the stability of individual proteins in the presence of defined deletion mutations[46]. Individual letters represent Dot protein names, whereas letters preceded by an 'i' indicate Icm protein names.

of many translocated substrates to the Dot/Icm secretion system[36,37]. In fact, binding to IcmS[38] or IcmW[39] has been used to identify substrates. Binding of IcmS, IcmW and/or LvgA[37–40] to translocated substrates seems to occur within a complex that includes at least two of these three T4SS components[36,38–40]. Although interactions between IcmW, IcmS, LvgA and their targets seem to be reminiscent of stable interactions between chaperones and substrates in type III secretion systems (T3SSs), the relationship between these proteins is almost certainly more complicated. There is probably a much larger steady-state pool of translocated substrates than of Dot/Icm components, which is consistent with a transient interaction during the course of secretion (similar to chaperone-assisted Sec-dependent secretion in bacteria[41]).

***The DotL–N translocation ATPase.*** The DotL protein shows strong sequence similarity to membrane-associated proteins that couple protein or DNA substrates to conjugative systems in preparation for transfer to target cells[42]. As there is evidence for direct binding of the ATPases to translocated substrates in several conjugative transfer systems[43,44], it is thought that proteins translocated by Dot/Icm bind to DotL, possibly using other Dot/Icm components as linkers. The crystal structure of one such coupling ATPase shows that the protein forms a hexameric ring, thereby providing a channel into which substrates can enter during transfer[45]. That DotL directly binds to DotM and DotN is suggested by the fact that the absence of one of these membrane proteins results in the degradation of the others. Furthermore, *dotL*$^-$, *dotM*$^-$ and *dotN*$^-$ mutants have similar phenotypes: mutations in each results in either sodium chloride hyper-sensitivity to the bacteria or lethality, depending on which strain harbours the mutations[46,47]. These proteins are also destabilized by the absence of IcmS or IcmW[47]. This suggests that a recognition site on the DotL–DotM–DotN membrane complex binds IcmW and/or IcmS proteins, which in turn bear substrates.

***The bacterial envelope-associated core complex.*** Much of the information that led to the concept of the core complex was based on the observation that stabilizing interactions occur between a subgroup of Dot/Icm proteins and that mutations in one of these components result in altered compartmentalization of the others. Five Dot/Icm components (DotC, DotD, DotF, DotG and DotH) interact to span the inner and outer bacterial membranes[47]. It is presumed that the most crucial outer-membrane partner is DotH, which fails to localize in the outer membrane in the absence of DotG or the outer-membrane lipoproteins DotC and DotD[47]. It is possible that DotH is the outer-membrane channel through which substrates pass as they transit from the DotF–DotG inner-membrane proteins through the DotL–DotM ATPase.

***Essential cytoplasmic components.*** Cytoplasmic components are necessary for the proper function of the Dot/Icm translocator. The cytoplasmic IcmQ–IcmR complex is a mysterious component of the translocation system[37,48]. The absence of either protein prevents translocation of substrates and formation of the replication vacuole, but there is no evidence for a direct interaction of either protein with any known membrane-associated protein. Although the IcmQ–IcmR complex might perform similar chaperone functions to those proposed for IcmW–IcmS, the phenotypes of mutations in the IcmQ–IcmR complex are not similar to those that affect IcmW–IcmS. As for mutants that lack membrane components, *icmQ*$^-$ or *icmR*$^-$ mutants cannot promote high multiplicity cytotoxicity in macrophages, an activity that is taken as an indicator for a functioning protein channel into target cells[37,48]. Consistent with the idea of channel formation, in the absence of IcmR, the IcmQ protein can insert into membranes[49]. However, as yet there is no evidence for Dot/Icm-dependent insertion of IcmQ into target membranes either after association of *L. pneumophila* with host cells or at any other stage of the life cycle[49].

***Inner-membrane accessory factors.*** IcmF and DotU (also known as IcmH) regulate the turnover of core components. Deletion mutations in *icmF* or *dotU* (also known as *icmH*) result in partial defects in intracellular growth and effector translocation, indicating that the products of these genes might support translocation[50,51]. In the absence of IcmF or DotU, the steady-state levels of DotG and DotH are reduced. Interestingly, IcmF and DotU are the most widely distributed of the Dot/Icm proteins: they have orthologues in many bacterial species that interact with host cells and lack recognizable T4SSs[52]. It has been argued that these orthologues are components of the recently discovered type VI secretion system (T6SS)[53].

Retrograde
The trafficking of vesicles in a direction that starts from the host cell surface and ends in the endoplasmic reticulum (ER); for example, traffic from the Golgi complex to the ER.

Autophagy
The process by which a cell degrades its own cytoplasmic constituents by packaging them into a membrane-bound compartment and fusing the resulting vacuole with the lysosome.

Table 1 | **Dot/Icm proteins**

| Protein | Comment and/or function |
|---|---|
| ***Substrate recognition*** | |
| IcmS[36–38,40] | Substrate recognition; presentation to translocon |
| IcmW[36–38,40] | Substrate recognition; presentation to translocon |
| LvgA[36] | Substrate recognition; presentation to translocon |
| ***Coupling ATPase*** | |
| DotL (also known as IcmO)[42,43] | ATPase; might bind directly to substrates |
| DotM (also known as IcmP)[46,47] | ATPase component |
| DotN (also known as IcmJ)[46,47] | Probable ATPase component |
| ***Core components*** | |
| DotC[47] | Putative outer-membrane lipoprotein |
| DotD[47] | Putative lipoprotein; localized to outer membrane |
| DotF (also known as IcmB)[47] | Interacts with substrates; might be a major component of a channel |
| DotG (also known as IcmE)[47] | Major component of a channel |
| DotH (also known as IcmK)[47] | Might be an outer-membrane channel |
| ***Core stability determinants*** | |
| DotU (also known as IcmH)[50,51] | Inner-membrane protein |
| IcmF[50,51] | Inner-membrane protein |
| ***Cytoplasmic components*** | |
| IcmQ[49] | Pore-forming molecule |
| IcmR[37,48] | Chaperone for IcmQ |
| DotB[54,117] | ATPase; might disassemble the translocon |
| DotO (also known as IcmB)[118] | Cytoplasm; inner-membrane protein |
| ***Inner membrane or periplasmic components of unknown function*** | |
| DotA[25,119] | Large polytopic inner-membrane protein |
| DotE (also known as IcmC)[47] | Similar to DotV |
| DotI (also known as IcmL)[118] | Inner membrane protein |
| DotJ (also known as IcmM) | Predicted inner-membrane protein |
| DotK (also known as IcmN)[120] | Predicted inner-membrane protein |
| DotP (also known as IcmD) | Predicted inner-membrane protein |
| DotV[47] | Predicted inner-membrane protein |
| IcmT[121] | Inner-membrane protein |
| IcmV[122] | Predicted inner-membrane protein |
| IcmX[123] | Periplasmic |

Dot/Icm, (defect in organelle trafficking/intracellular multiplication).

By analogy with the Dot/Icm system, the orthologues might not be directly involved in protein translocation, but instead might modulate the stability or function of the T6SS.

***Components of unknown function.*** The remaining proteins are generally essential for formation of the replication vacuole and intracellular growth, but their relationships with the other components are unknown (TABLE 1). The only hint regarding these proteins is based on the sequence similarity of DotB to PilT ATPases[54]. This family is associated with pili-promoted twitching motility, and can couple ATP hydrolysis in the cytoplasm to depolymerization of pili on the outer surface of the outer membrane. DotB might be involved in energy transfer across the bacterial envelope or in promoting disassembly of the complex at crucial points in the translocation process.

## Dot/Icm substrates

According to molecular Koch's postulate, originally formulated by Falkow[55], if a mutant can be shown to be defective for a crucial pathogenesis process, then the protein that is missing in the mutant can be called a virulence factor. The inability to detect a defect in a virulence-associated process has sometimes been used as an argument against the importance of a protein in disease. As emphasized by Falkow[56], this point of view is too simplistic, as many proteins have roles in pathogenesis that are too complex to be uncovered in the assays commonly used by investigators in the field. The analysis of the Dot/Icm substrates supports the complex view of the pathogen and highlights the difficulty in trying to formulate simple definitions of virulence factors. Although most of the *dot/icm* genes completely prevent the formation of replication vacuoles and intracellular growth, the substrates of Dot/Icm often fail this simple test for significance. The best-case scenario for some of the substrates is that their absence results in only partial defects in intracellular growth or replication-vacuole morphology[38,57,58]. As a result, screens for mutants that are defective in intracellular growth have only uncovered genes that encode a few substrates, the most profound being *sdhA* (*sidH* paralogue A). Deletion of *sdhA* generally blocks intracellular growth without affecting the formation of replication vacuoles[59] (discussed below). In addition to screening for defective intracellular growth, other strategies are therefore needed to identify the Dot/Icm substrates.

Several complementary strategies have been used to identify such substrates (BOX 1). The four main approaches are: the use of bioinformatics analysis to identify proteins that are likely to have activities only within eukaryotic cells[31,33,60–62]; the use of gene fusions to detect protein sequences that promote the translocation of an assayable protein fragment[27,32,63]; the identification of *L. pneumophila* proteins that disrupt cellular processes in the yeast *Saccharomyces cerevisiae*[57,64]; and the identification of regulatory networks that control translocated substrates[65] (TABLE 2; Supplementary information S1 (table)). To date, 85 proteins have been identified that contain a signal recognized by the Dot/Icm system (Supplementary information S1 (table)). Representatives of these substrates are shown in TABLE 2, and were chosen so that the substrates represent examples of most of the structural elements predicted by sequence analysis. In addition to the substrates in TABLE 2 and Supplementary information S1 (table), we have identified an additional 65 proteins with sequences that can provide translocation signals (data not shown). The number of substrates is likely to be much larger than this total of 140, as none of the strategies used to identify substrates has been performed in a saturating manner. In addition, the complete sequencing

Box 1 | **Searching for translocated substrates**

Translocated substrates of Dot/Icm (defect in organelle trafficking/intracellular multiplication) have been identified using four different methods (TABLE 2; Supplementary information S1 (table)). In the first strategy, bioinformatics picked out almost 50 potential substrates. These proteins have similar sequences to those involved in processes that are unique to eukaryotic cells[31,61,62]. These *leg* (*Legionella* eukaryotic-like) genes include kinases, lyases and esterases[31,61]. Several of these potential substrates are predicted to be involved in ubiquitination, and one was shown to be a ubiquitin ligase[67]. Furthermore, several dozen proteins with predicted coiled-coil secondary structures are encoded in the four sequenced *Legionella pneumophila* strains, as are proteins with ankyrin and leucine-rich repeats[31,61,62,73,113]. A second strategy identified biological regulatory networks that control identified substrates and extended the analysis to identify other genes that are similarly regulated[75].

A third strategy was to identify Dot/Icm substrates by the presence of translocation signals[27,32]. Such proteins (called Sid; substrates of Icm/Dot) were identified using a Cre–lox site assay, in which fusions were constructed between the 3′ ends of *L. pneumophila* genes and the Cre site-specific recombinase gene[114]. The recombinase fusions by the Dot/Icm system was detected by mixing the fusions strains with a recipient strain that had an antibiotic resistance detector read-out for acquisition of the recombinase[38,58,73].

A fourth strategy identified translocated proteins by screening for *Legionella* proteins that disrupt cellular functions when ectopically expressed in *Saccharomyces cerevisiae*[57,64] (TABLE 2). Proteins translocated by bacteria into host cells cause 'misregulation' of biochemical pathways in eukaryotic cells, which can be detected as growth defects in yeast[115]. Few, if any, proteins involved in bacterial housekeeping functions trigger such growth defects[116]. Shuman and co-workers[64] hunted specifically for proteins that could disrupt secretory function. Four such proteins, called Vips, were identified. Similarly, a general screen for loss of viability was performed by introducing a random bank of *L. pneumophila* genes into yeast[57]. This identified YlfA, which localizes to the early secretory apparatus, as well as SidE and SdcA (SidC paralogue A), which were identified using the Cre–Lox assay[73].

of several strains indicates that there may be substantial variation between different clinical isolates in the number of translocated substrates[30,31].

The wealth of Dot/Icm substrates should generate sufficient information to allow detection of a common motif recognized by the Dot/Icm apparatus (TABLE 2; Supplementary information S1 (table)). In fact, sequence patterns in known translocated substrates have allowed further bioinformatic identification of substrates. The T4SS seems to recognize a signal on the carboxyl (C) terminus of target proteins, and an analysis of the C terminus of RalF showed that a hydrophobic residue, two amino acids upstream of the C terminus, is crucial for translocation of RalF into mammalian cells[66]. Extending the analysis of known translocated substrates further, polar and small residues seem to be common upstream of the hydrophobic residue[67]. By looking for similar arrangements of sequences near the C termini of all *L. pneumophila* proteins, 19 more Dot/Icm substrates were identified that were not detected using other strategies[67]. The fact that only a subset of translocated substrates can be found using this strategy, however, underlines the difficulty of finding a single recognition signal for translocation.

## Regulation of translocated substrates

Many strains of *L. pneumophila* must be grown to post-exponential phase in broth culture to enable efficient intracellular replication after introduction into the host[68]. Consistent with this phenomenon, proteins involved in regulating post-exponential phase gene expression are required for optimal intracellular replication[69–72]. Furthermore, several of the translocated substrates of Dot/Icm are most highly expressed in post-exponential phase[33,58,73,74]. This indicates that common regulators might control many of the substrate-encoding genes. A consensus regulatory sequence (cTTAATatT) that seems to be recognized by PmrA, a two-component response regulator[65], is present upstream of several genes that encode Dot/Icm substrates. Many of these genes have reduced expression in the absence of PmrA, and a Δ*pmrA* strain is defective for intracellular growth, indicating that PmrA might control many proteins that interface with host cells. An additional 35 targets of PmrA were identified from the presence of the consensus sequence, several of which are linked on the chromosome to *dot/icm* substrate-encoding genes[65]. Several of these *ceg*s (coregulated with effector genes) have eukaryotic motifs. Furthermore, seven *ceg*s (Supplementary information S1 (table)) were shown to be translocated in a Dot/Icm-dependent manner using an enzymatic assay[65]. Similarly, nine translocated substrates were identified after searching for genes regulated by the CpxR transcriptional regulator[75].

## Modulation by Dot/Icm substrates

The replication vacuole hijacks host cell membrane material by recruiting host cell regulatory and effector proteins that promote vesicle budding, tethering and fusion throughout the early secretory system. The recruitment of Arf1, Rab1 and Sec22 (REFS 11,12) makes each of these proteins a potential target of the translocated substrates (FIG. 2). The observation that the translocated substrate RalF activates Arf1, and that *ralF*$^-$ mutants are defective for recruitment of Arf1 to the LCV, provided the first support for this idea[33]. However, these mutants can still grow intracellularly, even though chemical inhibition of Arf family function interferes with intracellular growth[14]. Therefore, although Arf1 activity is important for intracellular growth, its recruitment to the LCV is of unknown importance. Thus, either other *L. pneumophila* proteins manipulate Arf activity or host cell activators of Arf can regulate membrane trafficking processes that are important for intracellular growth.

The story of the recruitment of Rab1 to the LCV follows a similar scenario. Association of Rab1 with the LCV depends on the Dot/Icm translocated substrate SidM[76] (also known as DrrA[77]), which activates Rab1 by promoting nucleotide exchange. Reminiscent of the Arf1 story, dominant inhibitory variants of Rab1 interfere with LCV formation[11,12], so it might be expected that recruitment of Rab1 by SidM would be crucial for intracellular growth — but it is not. Mutants that lack SidM grow intracellularly in all cell types tested[76,77]. This lack of phenotype is particularly strange, given that *L. pneumophila* seems to encode many proteins that modulate Rab1 dynamics. Another translocated substrate, LidA, binds to Rab1 (as well as other Rab family members)[76], and a third translocated substrate, LepB, is a GTPase activating protein (GAP) for Rab1[78]. This indicates that *L. pneumophila* can control the complete cycle of Rab1 activation (through SidM) and

Table 2 | **Examples of Dot/Icm translocated substrates***

| Protein | Gene locus | Domain or function | Evidence for translocation |
|---|---|---|---|
| ***Substrates based on similarity to eukaryotic proteins*** | | | |
| RalF[33] | *lpg1950* | Sec7 homology domain and Arf1 GEF; Arf1 recruitment | Cya-fusion assay and immunofluorescence microscopy |
| LepA[62] | *lpg2793* | Homology to EEA1, USO1, SNAREs and coiled-coil domain; bacterial egress | Cya-fusion assay and fusions to β-lactamase[125] |
| LepB[62,78] | *lpg2490* | Homology to EEA1, USO1, SNAREs, coiled-coil domain and Rab1 GAP; vesicle trafficking and bacterial egress | Cya-fusion assay |
| LegA8 (also known as AnkN and AnkX)[61,63,124,] | *lpg0695* | Ankyrin repeat | Cya-fusion assay and fusions to β-lactamase[125] |
| LegAU13 (also known as Ceg27 and AnkB)[61,65,124] | *lpg2144* | F box; ankyrin repeat | Fusions to β-lactamase[125] |
| LegC8 (also known as Lgt2)[61] | *lpg2862* | Glucosyltransferase; coiled-coil domain | Fusions to β-lactamase[125] |
| LegL3 (REF. 61) | *lpg1660* | Leucine-rich repeat | Cya-fusion assay and fusions to β-lactamase[125] |
| LegLC8 (REF. 61) | *lpg1890* | Leucine-rich repeat; coiled-coil domain | Cya-fusion assay and fusions to β-lactamase[125] |
| LegG2 (REF. 61) | *lpg0276* | Ras GEF | Cya-fusion assay and fusions to β-lactamase[125] |
| LegP[31,61] | *lpg2999* | Astacin protease | Fusions to β-lactamase[125] |
| LegT[61] | *lpg1328* | Thaumatin domain | Fusions to β-lactamase[125] |
| LegU1 (REF. 61) | *lpg0171* | F box | Fusions to β-lactamase[125] |
| ***Substrates identified by directly assaying for Dot/Icm-dependent translocation*** | | | |
| SidF[39,73,81] | *lpg2584* | Bcl2-rambo and BNIP3 binding domain; anti-apoptosis | Inter-bacterial transfer, immunofluorescence microscopy and cya-fusion assay |
| SdhA[59] | *lpg0376* | Coiled-coil domain; anti-apoptosis | Saponin extraction |
| ***Substrates identified in yeast ectopic overexpression studies*** | | | |
| VipA[64] | *lpg0390* | Formin homology domain; vesicle trafficking | Cya-fusion assay |
| YlfA (also known as LegC7)[57,61] | *lpg2298* | Coiled-coil domain; vesicle trafficking | Cya-fusion assay and fusions to β-lactamase[125] |
| ***Substrates identified based on regulatory networks*** | | | |
| Ceg10 (REF. 65) | *lpg0284* | Hypothetical protein | Cya-fusion assay |
| ***Substrate identified by a putative Dot/Icm translocation signal*** | | | |
| Putative uncharacterized protein[67] | *lpg0045* | Hypothetical protein | Cya-fusion assay |
| ***Substrates identified by other mechanisms*** | | | |
| SidM (also known as DrrA)[76,77] | *lpg2464* | Rab1 GEF and Rab1 GDI; Rab1 recruitment | Cya-fusion assay and immunofluorescence microscopy |
| LidA[35,76] | *lpg0940* | Coiled-coil domain; Rab1 sequestration | Immunofluorescence microscopy and PNS |
| SidJ[80] | *lpg2155* | Endoplasmic reticulum recruitment | Saponin extraction and SidC-based translocation assay |
| WipA[39] | lpg2718 | Hypothetical protein | Cya-fusion assay |

*A complete list of the Dot/Icm translocated substrates is provided in Supplementary information S1 (table). Arf1, ADP-ribosylation factor 1; Bcl2, B-cell lymphoma 2; EEA1, early endosomal antigen 1; GAP, GTPase activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; PNS, protein present on phagosomes isolated from post-nuclear supernatants of infected cells; RalF, recruitment of Arf1 to *Legionella* phagosome; SdhA, *sidH* paralogue A; Sid, substrates of Icm/Dot.

inactivation (through LepB), and use a third protein for recognition. However, bacteria that lack the proteins which manipulate Rab1 have only small defects in establishing the LCV[79]. In fact, no effector of known activity has been shown to be an important component of LCV formation, although mutations in a previously uncharacterized protein, SidJ, have been shown to reduce ER recruitment[35,80].

The genetic analysis of translocated substrates has been frustrating, but the biochemistry of their activities has been fascinating. For example, SidM has a novel activity that has not been observed in other guanine nucleotide exchange factor (GEF) proteins. In eukaryotic cells, Rab GTPases are geranylgeranylated. In their inactive GDP-bound form, Rab proteins associate with Rab guanine nucleotide dissociation

inhibitor (GDI) proteins, which block the exposure of the Rab lipid tail to the aqueous environment and allow the formation of a soluble pool of GTPases[81]. This raises a problem for Rab GEF proteins: they are blocked from activating Rabs bound to GDI. There is evidence, at least in one case, that a GDI dissociation factor (GDF) can extract Rab proteins from the soluble pools[82]. Although this protein, called Pra1, might be involved in LCV formation, there is no reason that it should be necessary to extract and recruit Rab1 to the LCV. This is because SidM has both GEF and GDF activities, as it can extract and activate geranylgeranylated Rab1 (REFS 78,83). In a pure system, SidM can remove Rab1 from its GDI-bound partner and deliver activated protein to synthetic lipid vesicles, thereby reconstructing the entire recruitment process *in vitro*[83]. Furthermore, both the GDF and GEF activities of SidM are necessary for recruiting Rab1 to membranes in living cells, providing the only *in vivo* evidence that GDF activity is needed for the delivery and activation of a Rab protein to cellular membranes[78].

## Effector redundancy

Given that the Dot/Icm system is required for LCV formation, and the fact that four Dot/Icm substrates have activities that manipulate ER-to-Golgi traffic, it is likely that the translocated substrates have a role in promoting the formation of replication vacuoles[33,76,77,83]. The difficulty in detecting phenotypes of deletion mutations in genes for substrates may be indicative of functional redundancy, such that multiple proteins can carry out similar functions. This presents a difficult problem: few systematic approaches allow redundant functions to be identified. Inspection of four sequenced *L. pneumophila* genomes could provide insights, as many of the translocated substrates are members of protein families[30,31,73]. In some cases, substrates have as many as five paralogues; unfortunately, there is little evidence that deletion of all of the paralogues in a family reveals a new phenotype[38,57,58]. The only exceptions to this rule are the *lepA lepB* double mutant and the removal of all three paralogues of the *sdhA* family. The *lepA lepB* double mutant reveals a defect in lysis from amoebae[62], whereas a profound defect in host cell survival caused by loss of *sdhA* is exacerbated by loss of the other two paralogues[59].

Functional redundancy might occur if substrates target different host cell trafficking pathways that can each promote LCV formation. If so, eliminating one of these processes should cause the bacterium to become dependent on the remaining pathway (or pathways), thereby revealing phenotypes that are not otherwise apparent. Evidence for this model was obtained by replicating *L. pneumophila* in cells of *Drosophila melanogaster*[84]. On the one hand, interrupting individual membrane trafficking pathways, using RNA interference (RNAi) against specific components involved in vesicle budding and fusion, often results in little or no reduction in *L. pneumophila* intracellular growth. On the other hand, if RNAi is targeted against appropriate pairs of transcripts that encode proteins involved in different steps in membrane trafficking, defects in intracellular growth can be detected[84]. The *L. pneumophila* translocated substrates might therefore target each of these pathways, raising the possibility that interfering with the function of one of these pathways might allow phenotypes of bacterial mutants to be revealed. Similar redundancy might also be present in other intracellular pathogens, such as *Salmonella* serovars and *Shigella* species[85,86].

A comparison of *L. pneumophila* with the plant pathogen *Pseudomonas syringae*, which translocates proteins into host plant cells through a T3SS, could shed light on the high number of substrate-encoding genes in the *L. pneumophila* genome. As in *L. pneumophila*, hundreds of T3SS substrates encoded by *P. syringae* have been identified, but these substrates are distributed over a high number of pathogenic isolates[87,88]. Any single *P. syringae* isolate rarely has more than 40 known substrates[89]. These strains are highly adapted to a limited spectrum of hosts, so that host specificity is at least partially determined by the strain-specific spectrum of the T3SS substrates. By contrast, *L. pneumophila* is not a specialist in the same sense. Although *L. pneumophila* has adapted to grow in amoebae and other unicellular microorganisms, no amoebic host preference has been detected, and many cell types can support intracellular growth of this bacterium[90]. Although there might have been powerful selection for the acquisition or generation of new substrate genes to facilitate intracellular growth in multiple amoebic species, there has been less selective pressure for the loss of genes. Presumably, a set of genes that does not facilitate optimal growth in one host allows a selective advantage when the next species is encountered.

Although this model explains the lack of host specificity and the multitude of substrates, it does not completely explain functional redundancy, as one could imagine a pathogen in which loss of proteins that are optimized for growth in one host should result in a profound intracellular growth defect in that particular host. Although there is evidence that certain proteins in *L. pneumophila* selectively provide an advantage to the pathogen in certain hosts (for example, SdhA, SidF and SidJ)[59,80,91], for most substrates the consequences of deletions are subtle or nonexistent during the timescale of normal laboratory experiments. Translocated substrates that are optimal in one host might have only partial activities in another, thereby contributing to the appearance of redundancy. This model also predicts that because the main selection mechanism for a microorganism is to be a generalist, individual *L. pneumophila* strains do not need an identical spectrum of substrates, as long as the organism can grow in multiple hosts. The four completely sequenced strains are therefore predicted to have many substrates that are only present in a subset of strains[30,31].

## Survival of the host cell

Growth of *L. pneumophila* within macrophages involves a battle between life and death for the host cell. As continued intracellular replication requires a live macrophage, the bacterium must ensure the survival of the host cell against assault by toxic microbial products and the immune system. *L. pneumophila* can induce Dot/Icm-dependent death through both apoptotic[92–94] and non-apoptotic

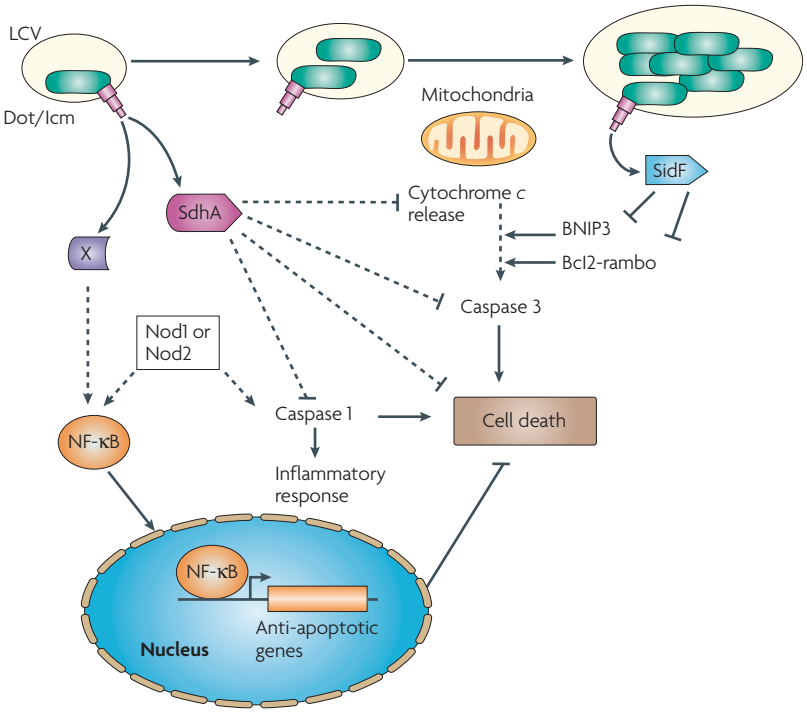


Figure 4 | ***Legionella pneumophila* manipulates host cell death and survival pathways**. After uptake into mammalian cells, a response to *L. pneumophila* that threatens to terminate intracellular growth by causing host cell death occurs. The cell death pathways have both a necrotic as well as an apoptotic character, and require an intact Dot/Icm (defect in organelle trafficking/intracellular multiplication) translocation system. The individual *L. pneumophila* components or translocated substrates that cause cell death have not been identified. In addition, there are at least two translocated substrates that interfere with host cell death. SdhA (*sidH* paralogue A) is required to inhibit multiple pathways that lead to cell death after *L. pneumophila* contact with host cells, and its absence causes a defect in intracellular replication within macrophages[59]. *L. pneumophila* also activates the host transcription factor nuclear factor-κB (NF-κB) to promote expression of anti-apoptotic genes to delay host cell death[102,103]. However, the mechanism by which this occurs has not yet been determined. At later stages of infection, SidF directly inhibits an apoptotic pathway by interfering with pro-death proteins in the rambo family[91]. Bcl2, B-cell lymphoma 2; LCV, *Legionella*-containing vacuole; Nod, nodulation; Sid, substrates of Icm/Dot.

pathways[94,95], whereas innate immune mechanisms can lead to premature death of infected macrophages, thereby terminating the replication cycle[96,97]. These events inhibit intracellular replication. Macrophage death caused by *L. pneumophila* can most clearly be observed under conditions of high bacterial loads, which results in the induction of caspase 3 (REFS 79,94), and in some cell types, caspase 1 (REFS 97,98). Although it has been argued that caspase 3 might support intracellular replication[99], the consensus is that the bacterium must interfere with caspase activation in some way to support intracellular growth[59,97]. In addition, high multiplicities of infection damage the host cell membrane, leading to cellular death[94,95], and similar types of non-apoptotic death are apparent even at low doses of bacteria[59]. Most of the microbial components that induce cell death have not been identified, although the bacterial flagellin protein seems to promote caspase 1 -dependent cell death in macrophages isolated from mouse strains that fail to support efficient growth of *L. pneumophila*[98,100].

Importantly, *L. pneumophila* can interfere with host cell death using a mechanism that requires the Dot/Icm translocator[101] (FIG. 4). The mechanisms that protect against host cell death are probably diverse, because many types of death pathway seem to be induced in mammalian cells in response to *L. pneumophila*. One strategy used by the bacterium is to induce transcription of host cell anti-apoptotic genes, at least some of which are positively regulated by the nuclear factor-κB transcription factor[102,103]. In addition, two translocated substrates of the Dot/Icm system interfere with host cell death. SidF interferes with specific pro-apoptotic pathways induced in response to *L. pneumophila*[91] by binding to two members of the Bcl2 (B-cell lymphoma 2) family of pro-apoptotic proteins, Bcl-rambo and BNIP3, and thereby interfering with an intrinsic death pathway that is initiated by these proteins[103,104]. Interestingly, SidF seems to be necessary for protection against host cell death only during the last few hours of intracellular replication, as Δ*sidF* mutants initiate replication efficiently and host cells that harbour the mutant are healthy during the first several hours of encounter[91].

A mutation that eliminates another translocated substrate, SdhA, has profound effects on intracellular growth in bone marrow-derived macrophages from mice: Δ*sdhA* mutants induce cell death shortly after uptake[59]. The fact that such a strong phenotype resulted from the loss of a translocated substrate is unique, and indicates that interference with cell death by SdhA is the main strategy used to promote host cell survival. SdhA is one of three paralogues expressed by *L. pneumophila* subsp. pneumophila str. Philadelphia 1, and deletion of all three genes results in an *L. pneumophila* strain that cannot replicate in bone marrow-derived macrophages. Although the mechanism of SdhA-dependent protection from host cell death has not been determined, it must either target a step that is common to a range of cell-death pathways or have multiple sites of action: both caspase-dependent and caspase-independent pathways of cell death are inhibited by bacteria that encode SdhA[59].

One striking phenotype of strains with *sidF* and *sdhA* knockout mutations is that growth defects for these mutants are only observed in macrophages. For most pathogens that are selected for growth on a particular mammalian host, there would be nothing odd about this result; however, for *L. pneumophila*, there is no explanation for the selective pressures that could have led to this specificity. According to current models, *L. pneumophila* is an 'accidental pathogen' in which selective pressures are directed towards evolving an organism that survives and grows efficiently within amoebae[105]. The fact that SidF binds two pro-death family members that are not found in lower eukaryotes cannot easily be explained by this theory. Either human pathogenic *L. pneumophila* strains have been selected for virulence by growth in a higher eukaryote or they encountered simple uncharacterized eukaryotes that have similar death cascades to those present in multicellular organisms, which would be consistent with the observation that programmed cell-death cascades occur in amoebae and involve apoptotic, necrotic and autophagy pathways[106–108].

## Conclusions

The intracellular life cycle of *L. pneumophila* is well characterized, and most of the mutants that have profound defects in establishing a replicative niche in the host cells have probably already been identified. Four complete genome sequences of related strains have been completed, allowing comparative analysis of substrates[30,31,109]. Many translocated proteins have also been identified in *L. pneumophila* subsp. *pneumophila* str. Philadelphia 1. However, it is difficult to show that any of the translocated effectors are essential in replication-vacuole biogenesis. Analysis of *L. pneumophila* pathogenesis is complicated because it is not a robust pathogen and high doses of bacteria are required to establish disease. Animals that are defective for Toll-like-receptor signalling are more susceptible to the pathogen[110], raising hope that novel animal infection models might provide new insights into the disease process. The fact that the related organism *Legionella longbeachae* causes severe disease in mice and therefore could be used in similar studies might be a partial solution, but this organism is not well characterized[111,112].

It might be possible to take a systems biology approach to probe how *L. pneumophila* grows within host cells. The four sequenced genomes contain blocks of dissimilarity, and isolated genes have been both lost and acquired, which might define regions that encode translocated substrates of Dot/Icm[30,31]. Analysing the members of the regulons controlled by CpxR, PmrA and RpoS could also provide information on host–pathogen interactions[65,71,72,75,89].

This is an exciting time to be studying the biology of *L. pneumophila* intracellular growth. Although the problems raised are complex, solutions to these problems are likely to be satisfying and could integrate data on the contributions of hundreds of different proteins to the formation of the replication vacuole.


1. Rowbotham, T. J. Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J. Clin. Pathol.* **33**, 1179–1183 (1980).
2. Muder, R. R., Yu, V. L. & Woo, A. H. Mode of transmission of *Legionella pneumophila*. A critical review. *Arch. Intern. Med.* **146**, 1607–1612 (1986).
3. Brieland, J. *et al.* Coinoculation with *Hartmannella vermiformis* enhances replicative *Legionella pneumophila* lung infection in a murine model of Legionnaires' disease. *Infect. Immun.* **64**, 2449–2456 (1996).
4. Segal, G. & Shuman, H. A. *Legionella pneumophila* utilizes the same genes to multiply within *Acanthamoeba castellanii* and human macrophages. *Infect. Immun.* **67**, 2117–2124 (1999).
5. Abu Kwaik, Y. The phagosome containing *Legionella pneumophila* within the protozoan *Hartmannella vermiformis* is surrounded by the rough endoplasmic reticulum. *Appl. Environ. Microbiol.* **62**, 2022–2028 (1996).
6. Horwitz, M. A. The Legionnaires' disease bacterium (*Legionella pneumophila*) inhibits phagosome–lysosome fusion in human monocytes. *J. Exp. Med.* **158**, 2108–2126 (1983).
7. Horwitz, M. A. Formation of a novel phagosome by the Legionnaires' disease bacterium (*Legionella pneumophila*) in human monocytes. *J. Exp. Med.* **158**, 1319–1331 (1983).
**Together with Reference 6, this early study showed that *L. pneumophila* replicates inside eukaryotic cells within a morphologically distinct membrane-bound vacuole that evades fusion with endocytic compartments.**
8. Horwitz, M. A. & Maxfield, F. R. *Legionella pneumophila* inhibits acidification of its phagosome in human monocytes. *J. Cell Biol.* **99**, 1936–1943 (1984).
9. Swanson, M. S. & Isberg, R. R. Association of *Legionella pneumophila* with the macrophage endoplasmic reticulum. *Infect. Immun.* **63**, 3609–3620 (1995).
**Electron-microscopy based study which provides evidence that the LCV is similar to the host cell ER in morphology and protein composition.**
10. Tilney, L. G., Harb, O. S., Connelly, P. S., Robinson, C. G. & Roy, C. R. How the parasitic bacterium *Legionella pneumophila* modifies its phagosome and transforms it into rough ER: implications for conversion of plasma membrane to the ER membrane. *J. Cell Sci.* **114**, 4637–4650 (2001).
11. Derre, I. & Isberg, R. R. *Legionella pneumophila* replication vacuole formation involves rapid recruitment of proteins of the early secretory system. *Infect. Immun.* **72**, 3048–3053 (2004).
12. Kagan, J. C., Stein, M. P., Pypaert, M. & Roy, C. R. *Legionella* subvert the functions of Rab1 and Sec22b to create a replicative organelle. *J. Exp. Med.* **199**, 1201–1211 (2004).
13. Lu, H. & Clarke, M. Dynamic properties of *Legionella*-containing phagosomes in *Dictyostelium* amoebae. *Cell. Microbiol.* **7**, 995–1007 (2005).
14. Kagan, J. C. & Roy, C. R. *Legionella* phagosomes intercept vesicular traffic from endoplasmic reticulum exit sites. *Nature Cell Biol.* **4**, 945–954 (2002).
15. Robinson, C. G. & Roy, C. R. Attachment and fusion of endoplasmic reticulum with vacuoles containing *Legionella pneumophila*. *Cell. Microbiol.* **8**, 793–805 (2006).
16. Sturgill-Koszycki, S. & Swanson, M. S. *Legionella pneumophila* replication vacuoles mature into acidic, endocytic organelles. *J. Exp. Med.* **192**, 1261–1272 (2000).
17. Wieland, H., Goetz, F. & Neumeister, B. Phagosomal acidification is not a prerequisite for intracellular multiplication of *Legionella pneumophila* in human monocytes. *J. Infect. Dis.* **189**, 1610–1614 (2004).
18. Molmeret, M., Bitar, D. M., Han, L. & Kwaik, Y. A. Disruption of the phagosomal membrane and egress of *Legionella pneumophila* into the cytoplasm during the last stages of intracellular infection of macrophages and *Acanthamoeba polyphaga*. *Infect. Immun.* **72**, 4040–4051 (2004).
19. Swanson, M. S. & Fernandez-Moreira, E. A microbial strategy to multiply in macrophages: the pregnant pause. *Traffic* **3**, 170–177 (2002).
20. Shintani, T. & Klionsky, D. J. Autophagy in health and disease: a double-edged sword. *Science* **306**, 990–995 (2004).
21. Levine, B. & Klionsky, D. J. Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev. Cell* **6**, 463–477 (2004).
22. Amer, A. O. & Swanson, M. S. Autophagy is an immediate macrophage response to *Legionella pneumophila*. *Cell. Microbiol.* **7**, 765–778 (2005).
23. Otto, G. P. *et al.* Macroautophagy is dispensable for intracellular replication of *Legionella pneumophila* in *Dictyostelium discoideum*. *Mol. Microbiol.* **51**, 63–72 (2004).
24. Marra, A., Blander, S. J., Horwitz, M. A. & Shuman, H. A. Identification of a *Legionella pneumophila* locus required for intracellular multiplication in human macrophages. *Proc. Natl Acad. Sci. USA* **89**, 9607–9611 (1992).
25. Berger, K. H., Merriam, J. J. & Isberg, R. R. Altered intracellular targeting properties associated with mutations in the *Legionella pneumophila dotA* gene. *Mol. Microbiol.* **14**, 809–822 (1994).
**Together with Reference 24, this paper identifies genes (*dot/icm*) that are required for both proper trafficking of the LCV and subsequent intracellular growth of *L. pneumophila*.**
26. Segal, G. & Shuman, H. A. Characterization of a new region required for macrophage killing by *Legionella pneumophila*. *Infect. Immun.* **65**, 5057–5066 (1997).
27. Vogel, J. P., Andrews, H. L., Wong, S. K. & Isberg, R. R. Conjugative transfer by the virulence system of *Legionella pneumophila*. *Science* **279**, 873–876 (1998).
**Provides evidence that the *dot/icm* genes encode a secretion system that can transfer plasmid DNA between bacterial cells.**
28. Sadosky, A. B., Wiater, L. A. & Shuman, H. A. Identification of *Legionella pneumophila* genes required for growth within and killing of human macrophages. *Infect. Immun.* **61**, 5361–5373 (1993).
29. Komano, T., Yoshida, T., Narahara, K. & Furuya, N. The transfer region of IncI1 plasmid R64: similarities between R64 *tra* and *Legionella icm/dot* genes. *Mol. Microbiol.* **35**, 1348–1359 (2000).
30. Chien, M. *et al.* The genomic sequence of the accidental pathogen *Legionella pneumophila*. *Science* **305**, 1966–1968 (2004).
31. Cazalet, C. *et al.* Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nature Genet.* **36**, 1165–1173 (2004).
32. Segal, G., Purcell, M. & Shuman, H. A. Host cell killing and bacterial conjugation require overlapping sets of genes within a 22-kb region of the *Legionella pneumophila* genome. *Proc. Natl Acad. Sci. USA* **95**, 1669–1674 (1998).
33. Nagai, H., Kagan, J. C., Zhu, X., Kahn, R. A. & Roy, C. R. A bacterial guanine nucleotide exchange factor activates ARF on *Legionella* phagosomes. *Science* **295**, 679–682 (2002).
**First study to show that *L. pneumophila* targets a host cell vesicle-trafficking factor through the action of a Dot/Icm translocated substrate.**
34. Chardin, P. *et al.* A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. *Nature* **384**, 481–484 (1996).
35. Conover, G. M., Derre, I., Vogel, J. P. & Isberg, R. R. The *Legionella pneumophila* LidA protein: a translocated substrate of the Dot/Icm system associated with maintenance of bacterial integrity. *Mol. Microbiol.* **48**, 305–321 (2003).
36. Vincent, C. D. & Vogel, J. P. The *Legionella pneumophila* IcmS–LvgA protein complex is important for Dot/Icm-dependent intracellular growth. *Mol. Microbiol.* **61**, 596–613 (2006).
37. Coers, J. *et al.* Identification of Icm protein complexes that play distinct roles in the biogenesis of an organelle permissive for *Legionella pneumophila* intracellular growth. *Mol. Microbiol.* **38**, 719–736 (2000).
38. Bardill, J. P., Miller, J. L. & Vogel, J. P. IcmS-dependent translocation of SdeA into macrophages by the *Legionella pneumophila* type IV secretion system. *Mol. Microbiol.* **56**, 90–103 (2005).
39. Ninio, S., Zuckman-Cholon, D. M., Cambronne, E. D. & Roy, C. R. The *Legionella* IcmS–IcmW protein complex is important for Dot/Icm-mediated protein translocation. *Mol. Microbiol.* **55**, 912–926 (2005).
40. Cambronne, E. D. & Roy, C. R. The *Legionella pneumophila* IcmSW complex interacts with multiple Dot/Icm effectors to facilitate type IV translocation. *PLoS Pathog.* **3**, e188 (2007).
41. Rusch, S. L. & Kendall, D. A. Interactions that drive Sec-dependent bacterial protein transport. *Biochemistry* **46**, 9665–9673 (2007).

42. Gomis-Ruth, F. X., Sola, M., de la Cruz, F. & Coll, M. Coupling factors in macromolecular type-IV secretion machineries. *Curr. Pharm. Des.* **10**, 1551–1565 (2004).
43. Lu, J. & Frost, L. S. Mutations in the C-terminal region of TraM provide evidence for *in vivo* TraM–TraD interactions during F-plasmid conjugation. *J. Bacteriol.* **187**, 4767–4773 (2005).
44. Atmakuri, K., Ding, Z. & Christie, P. J. VirE2, a type IV secretion substrate, interacts with the VirD4 transfer protein at cell poles of *Agrobacterium tumefaciens*. *Mol. Microbiol.* **49**, 1699–1713 (2003).
45. Yeo, H. J., Savvides, S. N., Herr, A. B., Lanka, E. & Waksman, G. Crystal structure of the hexameric traffic ATPase of the *Helicobacter pylori* type IV secretion system. *Mol. Cell* **6**, 1461–1472 (2000).
46. Buscher, B. A. *et al.* The DotL protein, a member of the TraG-coupling protein family, is essential for viability of *Legionella pneumophila* strain Lp02. *J. Bacteriol.* **187**, 2927–2938 (2005).
47. Vincent, C. D. *et al.* Identification of the core transmembrane complex of the *Legionella* Dot/Icm type IV secretion system. *Mol. Microbiol.* **62**, 1278–1291 (2006).
**Thorough study that detected distinct subcomplexes in the Dot/Icm translocon.**
48. Dumenil, G. & Isberg, R. R. The *Legionella pneumophila* IcmR protein exhibits chaperone activity for IcmQ by preventing its participation in high-molecular-weight complexes. *Mol. Microbiol.* **40**, 1113–1127 (2001).
49. Dumenil, G., Montminy, T. P., Tang, M. & Isberg, R. R. IcmR-regulated membrane insertion and efflux by the *Legionella pneumophila* IcmQ protein. *J. Biol. Chem.* **279**, 4686–4695 (2004).
50. VanRheenen, S. M., Dumenil, G. & Isberg, R. R. IcmF and DotU are required for optimal effector translocation and trafficking of the *Legionella pneumophila* vacuole. *Infect. Immun.* **72**, 5972–5982 (2004).
51. Sexton, J. A., Miller, J. L., Yoneda, A., Kehl-Fie, T. E. & Vogel, J. P. *Legionella pneumophila* DotU and IcmF are required for stability of the Dot/Icm complex. *Infect. Immun.* **72**, 5983–5992 (2004).
52. Das, S., Chakrabortty, A., Banerjee, R., Roychoudhury, S. & Chaudhuri, K. Comparison of global transcription responses allows identification of *Vibrio cholerae* genes differentially expressed following infection. *FEMS Microbiol. Lett.* **190**, 87–91 (2000).
53. Pukatzki, S. *et al.* Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc. Natl Acad. Sci. USA* **103**, 1528–1533 (2006).
54. Wall, D. & Kaiser, D. Type IV pili and cell motility. *Mol. Microbiol.* **32**, 1–10 (1999).
55. Falkow, S. Molecular Koch's postulates applied to microbial pathogenicity. *Rev. Infect. Dis.* **10** (Suppl. 2), 274–276 (1988).
56. Falkow, S. Molecular Koch's postulates applied to bacterial pathogenicity — a personal recollection 15 years later. *Nature Rev. Microbiol.* **2**, 67–72 (2004).
57. Campodonico, E. M., Chesnel, L. & Roy, C. R. A yeast genetic system for the identification and characterization of substrate proteins transferred into host cells by the *Legionella pneumophila* Dot/Icm system. *Mol. Microbiol.* **56**, 918–933 (2005).
58. VanRheenen, S. M., Luo, Z. Q., O'Connor, T. & Isberg, R. R. Members of a *Legionella pneumophila* family of proteins with ExoU (phospholipase A) active sites are translocated to target cells. *Infect. Immun.* **74**, 3597–3606 (2006).
59. Laguna, R. K., Creasey, E. A., Li, Z., Valtz, N. & Isberg, R. R. A *Legionella pneumophila*-translocated substrate that is required for growth within macrophages and protection from host cell death. *Proc. Natl Acad. Sci. USA* **103**, 18745–18750 (2006).
60. Casanova, J. E. Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. *Traffic* **8**, 1476–1485 (2007).
61. de Felipe, K. S. *et al.* Evidence for acquisition of *Legionella* type IV secretion substrates via interdomain horizontal gene transfer. *J. Bacteriol.* **187**, 7716–7726 (2005).
62. Chen, J. *et al. Legionella* effectors that promote nonlytic release from protozoa. *Science* **303**, 1358–1361 (2004).
63. Pan, X., Luhrmann, A., Satoh, A., Laskowski-Arce, M. A. & Roy, C. R. Ankyrin repeat proteins comprise a diverse family of bacterial type IV effectors. *Science* **320**, 1651–1654 (2008).
64. Shohdy, N., Efe, J. A., Emr, S. D. & Shuman, H. A. Pathogen effector protein screening in yeast identifies *Legionella* factors that interfere with membrane trafficking. *Proc. Natl Acad. Sci. USA* **102**, 4866–4871 (2005).
65. Zusman, T. *et al.* The response regulator PmrA is a major regulator of the *icm/dot* type IV secretion system in *Legionella pneumophila* and *Coxiella burnetii*. *Mol. Microbiol.* **63**, 1508–1523 (2007).
66. Nagai, H. *et al.* A C-terminal translocation signal required for Dot/Icm-dependent delivery of the *Legionella* RalF protein to host cells. *Proc. Natl Acad. Sci. USA* **102**, 826–831 (2005).
67. Kubori, T., Hyakutake, A. & Nagai, H. *Legionella* translocates an E3 ubiquitin ligase that has multiple U-boxes with distinct functions. *Mol. Microbiol.* **67**, 1307–1319 (2008).
68. Byrne, B. & Swanson, M. S. Expression of *Legionella pneumophila* virulence traits in response to growth conditions. *Infect. Immun.* **66**, 3029–3034 (1998).
69. Hales, L. M. & Shuman, H. A. The *Legionella pneumophila rpoS* gene is required for growth within *Acanthamoeba castellanii*. *J. Bacteriol.* **181**, 4879–4889 (1999).
70. Bachman, M. A. & Swanson, M. S. RpoS co-operates with other factors to induce *Legionella pneumophila* virulence in the stationary phase. *Mol. Microbiol.* **40**, 1201–1214 (2001).
71. Hammer, B. K., Tateda, E. S. & Swanson, M. S. A two-component regulator induces the transmission phenotype of stationary-phase *Legionella pneumophila*. *Mol. Microbiol.* **44**, 107–118 (2002).
72. Tiaden, A. *et al.* The *Legionella pneumophila* response regulator LqsR promotes host cell interactions as an element of the virulence regulatory network controlled by RpoS and LetA. *Cell. Microbiol.* **9**, 2903–2920 (2007).
73. Luo, Z. Q. & Isberg, R. R. Multiple substrates of the *Legionella pneumophila* Dot/Icm system identified by interbacterial protein transfer. *Proc. Natl Acad. Sci. USA* **101**, 841–846 (2004).
74. Bruggemann, H. *et al.* Virulence strategies for infecting phagocytes deduced from the *in vivo* transcriptional program of *Legionella pneumophila*. *Cell. Microbiol.* **8**, 1228–1240 (2006).
75. Altman, E. & Segal, G. The response regulator CpxR directly regulates the expression of several *Legionella pneumophila icm/dot* components as well as new translocated substrates. *J. Bacteriol.* **190**, 1985–1996 (2008).
76. Machner, M. P. & Isberg, R. R. Targeting of host Rab GTPase function by the intravacuolar pathogen *Legionella pneumophila*. *Dev. Cell* **11**, 47–56 (2006).
77. Murata, T. *et al.* The *Legionella pneumophila* effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nature Cell Biol.* **8**, 971–977 (2006).
78. Ingmundson, A., Delprato, A., Lambright, D. G. & Roy, C. R. *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. *Nature* **450**, 365–369 (2007).
79. Neumeister, B., Faigle, M., Lauber, K., Northoff, H. & Wesselborg, S. *Legionella pneumophila* induces apoptosis via the mitochondrial death pathway. *Microbiology* **148**, 3639–3650 (2002).
80. Liu, Y. & Luo, Z. Q. The *Legionella pneumophila* effector SidJ is required for efficient recruitment of endoplasmic reticulum proteins to the bacterial phagosome. *Infect. Immun.* **75**, 592–603 (2007).
81. Goody, R. S., Rak, A. & Alexandrov, K. The structural and mechanistic basis for recycling of Rab proteins between membrane compartments. *Cell. Mol. Life Sci.* **62**, 1657–1670 (2005).
82. Sivars, U., Aivazian, D. & Pfeffer, S. R. Yip3 catalyses the dissociation of endosomal Rab–GDI complexes. *Nature* **425**, 856–859 (2003).
83. Machner, M. P. & Isberg, R. R. A bifunctional bacterial protein links GDI displacement to Rab1 activation. *Science* **318**, 974–977 (2007).
84. Dorer, M. S., Kirton, D., Bader, J. S. & Isberg, R. R. RNA interference analysis of *Legionella* in *Drosophila* cells: exploitation of early secretory apparatus dynamics. *PLoS Pathog.* **2**, e34 (2006).
85. Zhou, D., Chen, L. M., Hernandez, L., Shears, S. B. & Galan, J. E. A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization. *Mol. Microbiol.* **39**, 248–259 (2001).
86. Deiwick, J. *et al.* The translocated *Salmonella* effector proteins SseF and SseG interact and are required to establish an intracellular replication niche. *Infect. Immun.* **74**, 6965–6972 (2006).
87. Chang, J. H. *et al.* A high-throughput, near-saturating screen for type III effector genes from *Pseudomonas syringae*. *Proc. Natl Acad. Sci. USA* **102**, 2549–2554 (2005).
88. Vinatzer, B. A., Jelenska, J. & Greenberg, J. T. Bioinformatics correctly identifies many type III secretion substrates in the plant pathogen *Pseudomonas syringae* and the biocontrol isolate *P. fluorescens* SBW25. *Mol. Plant Microbe Interact.* **18**, 877–888 (2005).
89. Sarkar, S. F., Gordon, J. S., Martin, G. B. & Guttman, D. S. Comparative genomics of host-specific virulence in *Pseudomonas syringae*. *Genetics* **174**, 1041–1056 (2006).
90. Dorer, M. S. & Isberg, R. R. Non-vertebrate hosts in the analysis of host–pathogen interactions. *Microbes Infect.* **8**, 1637–1646 (2006).
91. Banga, S. *et al. Legionella pneumophila* inhibits macrophage apoptosis by targeting pro-death members of the Bcl2 protein family. *Proc. Natl Acad. Sci. USA* **104**, 5121–5126 (2007).
92. Muller, A., Hacker, J. & Brand, B. C. Evidence for apoptosis of human macrophage-like HL-60 cells by *Legionella pneumophila* infection. *Infect. Immun.* **64**, 4900–4906 (1996).
93. Gao, L. Y. & Abu Kwaik, Y. Apoptosis in macrophages and alveolar epithelial cells during early stages of infection by *Legionella pneumophila* and its role in cytopathogenicity. *Infect. Immun.* **67**, 862–870 (1999).
94. Gao, L. Y. & Abu Kwaik, Y. Activation of caspase 3 during *Legionella pneumophila*-induced apoptosis. *Infect. Immun.* **67**, 4886–4894 (1999).
95. Kirby, J. E., Vogel, J. P., Andrews, H. L. & Isberg, R. R. Evidence for pore-forming ability by *Legionella pneumophila*. *Mol. Microbiol.* **27**, 323–336 (1998).
96. Derre, I. & Isberg, R. R. Macrophages from mice with the restrictive *Lgn1* allele exhibit multifactorial resistance to *Legionella pneumophila*. *Infect. Immun.* **72**, 6221–6229 (2004).
97. Zamboni, D. S. *et al.* The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nature Immunol.* **7**, 318–325 (2006).
98. Molofsky, A. B. *et al.* Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. *J. Exp. Med.* **203**, 1093–1104 (2006).
99. Molmeret, M. *et al.* Activation of caspase-3 by the Dot/Icm virulence system is essential for arrested biogenesis of the *Legionella*-containing phagosome. *Cell. Microbiol.* **6**, 33–48 (2004).
100. Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F. & Vance, R. E. Flagellin-deficient *Legionella* mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog.* **2**, e18 (2006).
101. Abu-Zant, A. *et al.* Anti-apoptotic signalling by the Dot/Icm secretion system of *L. pneumophila*. *Cell. Microbiol.* **9**, 246–264 (2007).
102. Losick, V. P. & Isberg, R. R. NF-κB translocation prevents host cell death after low-dose challenge by *Legionella pneumophila*. *J. Exp. Med.* **203**, 2177–2189 (2006).
103. Webster, K. A., Graham, R. M. & Bishopric, N. H. BNip3 and signal-specific programmed death in the heart. *J. Mol. Cell. Cardiol.* **38**, 35–45 (2005).
104. Lee, H. & Paik, S. G. Regulation of BNIP3 in normal and cancer cells. *Mol. Cells* **21**, 1–6 (2006).
105. Bruggemann, H., Cazalet, C. & Buchrieser, C. Adaptation of *Legionella pneumophila* to the host environment: role of protein secretion, effectors and eukaryotic-like proteins. *Curr. Opin. Microbiol.* **9**, 86–94 (2006).
106. Arnoult, D. *et al.* On the evolutionary conservation of the cell death pathway: mitochondrial release of an apoptosis-inducing factor during *Dictyostelium discoideum* cell death. *Mol. Biol. Cell* **12**, 3016–3030 (2001).
107. Lam, D. & Golstein, P. A specific pathway inducing autophagic cell death is marked by an IP3R mutation. *Autophagy* **4**, 349–350 (2008).
108. Tresse, E., Kosta, A., Luciani, M. F. & Golstein, P. From autophagic to necrotic cell death in *Dictyostelium*. *Semin. Cancer Biol.* **17**, 94–100 (2007).
109. Glockner, G. *et al.* Identification and characterization of a new conjugation/type IVA secretion system (*trb/tra*) of *Legionella pneumophila* Corby localized on two mobile genomic islands. *Int. J. Med. Microbiol.* **298**, 411–428 (2008).

110. Archer, K. A. & Roy, C. R. MyD88-dependent responses involving Toll-like receptor 2 are important for protection and clearance of *Legionella pneumophila* in a mouse model of Legionnaires' disease. *Infect. Immun.* **74**, 3325–3333 (2006).
111. Asare, R. & Abu Kwaik, Y. Early trafficking and intracellular replication of *Legionella longbeachaea* within an ER-derived late endosome-like phagosome. *Cell. Microbiol.* **9**, 1571–1587 (2007).
112. Alli, O. A., Zink, S., von Lackum, N. K. & Abu-Kwaik, Y. Comparative assessment of virulence traits in *Legionella* spp. *Microbiology* **149**, 631–641 (2003).
113. Graumann, P. L. SMC proteins in bacteria: condensation motors for chromosome segregation? *Biochimie* **83**, 53–59 (2001).
114. Vergunst, A. C. *et al.* VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. *Science* **290**, 979–982 (2000).
115. Lesser, C. F. & Miller, S. I. Expression of microbial virulence proteins in *Saccharomyces cerevisiae* models mammalian infection. *EMBO J.* **20**, 1840–1849 (2001).
116. Slagowski, N. L., Kramer, R. W., Morrison, M. F., LaBaer, J. & Lesser, C. F. A functional genomic yeast screen to identify pathogenic bacterial proteins. *PLoS Pathog.* **4**, e9 (2008).
117. Wolfgang, M., van Putten, J. P., Hayes, S. F., Dorward, D. & Koomey, M. Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili. *EMBO J.* **19**, 6408–6418 (2000).
118. Andrews, H. L., Vogel, J. P. & Isberg, R. R. Identification of linked *Legionella pneumophila* genes essential for intracellular growth and evasion of the endocytic pathway. *Infect. Immun.* **66**, 950–958 (1998).
119. Brand, B. C., Sadosky, A. B. & Shuman, H. A. The *Legionella pneumophila icm* locus: a set of genes required for intracellular multiplication in human macrophages. *Mol. Microbiol.* **14**, 797–808 (1994).
120. Yerushalmi, G., Zusman, T. & Segal, G. Additive effect on intracellular growth by *Legionella pneumophila* Icm/Dot proteins containing a lipobox motif. *Infect. Immun.* **73**, 7578–7587 (2005).
121. Bitar, D. M., Molmeret, M. & Kwaik, Y. A. Structure–function analysis of the C-terminus of IcmT of *Legionella pneumophila* in pore formation-mediated egress from macrophages. *FEMS Microbiol. Lett.* **242**, 177–184 (2005).
122. Gal-Mor, O. & Segal, G. Identification of CpxR as a positive regulator of *icm* and *dot* virulence genes of *Legionella pneumophila*. *J. Bacteriol.* **185**, 4908–4919 (2003).
123. Matthews, M. & Roy, C. R. Identification and subcellular localization of the *Legionella pneumophila* IcmX protein: a factor essential for establishment of a replicative organelle in eukaryotic host cells. *Infect. Immun.* **68**, 3971–3982 (2000).
124. Habyarimana, F. *et al.* Role for the ankyrin eukaryotic-like genes of *Legionella pneumophila* in parasitism of protozoan hosts and human macrophages. *Environ. Microbiol.* **10**, 1460–1474 (2008).
125. de Felipe, K. S. *et al.* Legionella eukaryotic-like type IV substrates interfere with organelle trafficking. *PLoS Pathog.* **4**, e1000117 (2008).



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