

# The *Coxiella burnetii* Dot/Icm System Creates a Comfortable Home through Lysosomal Renovation

Hayley J. Newton and Craig R. Roy

Section of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut, USA

**ABSTRACT** Understanding the molecular pathogenesis of *Coxiella burnetii*, the causative agent of human Q fever, has historically been hindered by the technical difficulties of genetically manipulating obligate intracellular bacteria. The recent development of culture conditions suitable for axenic propagation of *C. burnetii* has paved the way for the application of a range of genetic techniques to address key questions within the field. Recent studies using mutational analysis have revealed that the *C. burnetii* Dot/Icm type 4 secretion system (T4SS) is an important virulence determinant that is essential for renovation of a lysosome into a mature *Coxiella*-containing vacuole (CCV) permissive of intracellular replication. Interestingly, a mutant of *C. burnetii* deficient in Dot/Icm function was found to be capable of replicating within the parasitophorous vacuole created by *Leishmania amazonensis*, which indicates that *C. burnetii* replication is not dependent on the cohort of Dot/Icm effector proteins *per se* but rather that the collective actions of effectors are required to create the specialized niche supportive of replication. Thus, a role for the Dot/Icm T4SS during the intracellular life cycle of *C. burnetii* has been more clearly defined by these studies, which demonstrate that advances in genetic analysis should allow future studies to focus on the intricacies of Dot/Icm effector functions that facilitate development of the unique CCV.

Many intracellular bacterial pathogens have specialized secretion systems that deliver into host cells a specific catalogue of effector proteins that utilize a wide range of mechanisms to modulate cellular functions. Host cell manipulation allows the bacteria to model a specific intracellular niche compatible with bacterial replication. A sophisticated example of this is the type 4 secretion system (T4SS) termed Dot and Icm (1). The intracellular pathogens *C. burnetii* and *Legionella pneumophila* encode functionally analogous versions of the Dot/Icm system (2, 3). It is well established that the Dot/Icm system is essential for the ability of *L. pneumophila* to modulate transport of the vacuole in which the pathogen resides to evade lysosomal killing and create a specialized organelle derived from the endoplasmic reticulum (4). Comprehensive investigations of the importance of the Dot/Icm system in the intracellular survival and establishment of the unique *Legionella*-containing vacuole suggested that the Dot/Icm system is also a crucial virulence feature of *C. burnetii*. This hypothesis provided the impetus for several studies that aimed to identify and characterize putative effector proteins delivered into host cells by the *C. burnetii* Dot/Icm system, and over 60 different *C. burnetii* proteins have now been identified as translocated substrates of the Dot/Icm system (5–10). Yet, until recently, definitive roles for the *C. burnetii* Dot/Icm system during infection had not been demonstrated.

The development of a complex nutrient medium, acidified citrate cysteine medium (ACCM), and of culture parameters that mimic the low pH and reduced oxygen conditions within the CCV has resulted in the creation of conditions suitable for axenic growth of *C. burnetii* (11, 12). Axenic cultivation has facilitated the development of genetic tools to allow researchers in the field to finally address key questions regarding the pathogenesis and intracellular propagation of *C. burnetii* (13, 14). These advances enabled Beare et al. to use a genetic approach to address the importance of the Dot/Icm system during intracellular infection, and the results were presented in the July/August 2011 issue of *mBio* (5). The authors of that study isolated a *C. burnetii* mutant with a transposon insertion in *icmD*, a gene predicted to encode an es-

sential inner membrane component of the Dot/Icm system. Analysis of the interaction between the *icmD* mutant and macrophage-like THP-1 cells has demonstrated that a functional Dot/Icm system is required for the translocation of Dot/Icm substrates, including the plasmid-encoded proteins CpeD and CpeE. Importantly, Dot/Icm function was found to be necessary for development of the large CCV, productive intracellular replication, and apoptosis protection of infected THP-1 cells. These data correlate nicely with those from another recent study demonstrating that a transposon insertion in the *C. burnetii icmL* gene abolished effector translocation and intracellular replication of *C. burnetii* in mammalian cells (6). Both studies demonstrated that the *C. burnetii* Dot/Icm system is not required for axenic growth or uptake by host cells.

Beare et al. developed an elegant genetic technique to demonstrate complementation of the *icmD* mutant that entailed using a Tn7-based transposon system to introduce the *icmDJB* operon into the chromosome in the *glmS*-CBU1788 intergenic region (5). Placing this operon under the control of an anhydrotetracycline-inducible promoter allowed investigators to examine the temporal requirements for expression of the Dot/Icm system. Strikingly, replication of the *icmD* mutant strain was achieved when *icmDJB* expression was induced 24 h after infection. This finding demonstrates that, in contrast to *L. pneumophila*, which requires translocation of effectors by the Dot/Icm system within minutes of uptake to promote vacuole remodeling prior to endocytic maturation (15), *C. burnetii* has adapted to withstand the antimicrobial activities of a lysosomal environment and can retain intracellular

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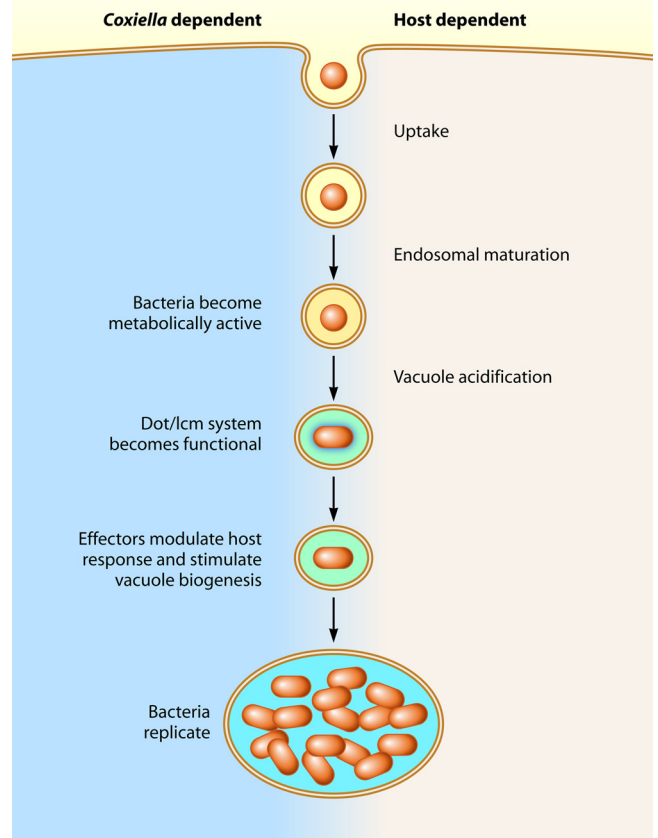
Address correspondence to Craig R. Roy, craig.roy@yale.edu.

viability within a nonpermissive lysosome independently of Dot/Icm function. Thus, the Dot/Icm system can somehow change the nature of a phagolysosome to make it permissive for *C. burnetii* replication.

Characterization of *C. burnetii* Dot/Icm-deficient strains has allowed the comparison and contrast of both the temporal requirements and functional outcomes of the Dot/Icm systems in *L. pneumophila* and *C. burnetii*. There are key features shared by these two systems; for example, both *L. pneumophila* and *C. burnetii* Dot/Icm-deficient strains can replicate in vacuoles shared by Dot/Icm-competent counterparts (5, 16), which highlights the observation that the effectors translocated by the Dot/Icm system are needed to create a vacuolar environment that is generally permissive for replication of the bacteria. Genome analysis of *L. pneumophila* and *C. burnetii* revealed extensive plasticity in the effectors produced by different strains of these organisms (17, 18), suggesting that, as with *L. pneumophila* (19), a significant degree of functional redundancy is likely built into the *C. burnetii* Dot/Icm substrate repertoire.

Comparative analysis revealed informative differences between the two Dot/Icm systems. The *L. pneumophila* Dot/Icm system is needed for the bacteria to immediately subvert host membrane transport pathways; because of this, the Dot/Icm system of *L. pneumophila* initiates effector translocation as soon as the extracellular bacteria make intimate contact with the plasma membrane of the host cell (20, 21). Because *C. burnetii* can utilize the host machinery for uptake and transport of the vacuole in which it resides through the endocytic pathway (22, 23), the bacteria should not need Dot/Icm-dependent functions to direct early membrane transport (Fig. 1). Consistent with this hypothesis, it appears that the *C. burnetii* Dot/Icm system is not functional until the bacteria have established residence in an acidified lysosome-derived vacuole several hours after uptake (6). *L. pneumophila* studies have shown that the ability of the bacteria to use the Dot/Icm system to communicate with the host cytosol to direct membrane transport leads to rapid detection by mammalian pattern recognition receptors in the cytosol that trigger a very robust innate immune response (24, 25). Thus, it is tempting to speculate that *C. burnetii* has evolved mechanisms to regulate the Dot/Icm system so that it does not deliver effectors during early endocytic transport stages; in this way, the bacteria may avoid innate immune signaling pathways that operate to detect pathogens with specialized secretion systems during the invasion process.

The finding that the *icmD* mutant is able to replicate in a *Leishmania amazonensis* vacuole is both intriguing and enlightening. It was observed previously that *C. burnetii* and the trypanosomatid parasite *L. amazonensis* are able to productively inhabit the same vacuole (26). Here, the ability of Dot/Icm-deficient *C. burnetii* to replicate in the complete absence of the *C. burnetii* repertoire of Dot/Icm effector proteins implies that the environment within the lumen of the *L. amazonensis* parasitophorous vacuole must closely mimic that of the CCV and/or of ACCM and that the Dot/Icm-dependent activities needed to promote replication of *C. burnetii* are provided by *L. amazonensis*. It is clear that both *L. amazonensis* and *C. burnetii* do not alter the lysosomal pH or hydrolytic capacity of their respective replicative vacuoles and that both pathogens reside in fusogenic vacuoles reminiscent of a lysosome with a low luminal pH. It is plausible that *L. amazonensis* and Dot/Icm-competent *C. burnetii* both mediate luminal alterations to either



**FIG 1** Host and pathogen functions important for *C. burnetii* infection. Infection by *C. burnetii* is initiated through subversion of host cellular processes. Small-cell variants of *C. burnetii* (red circles) that are metabolically inactive represent the infectious forms internalized by host cells. Host cellular processes direct endocytosis of attached bacteria, and the resulting CCV matures along the endocytic pathway through sequential membrane fusion events. Acidification of the vacuole is a signal that stimulates *C. burnetii* to become metabolically active and develop into replicative forms called large-cell variants (red ovals). It is at this stage that translocation of bacterial effector proteins into the host cell mediated by the Dot/Icm T4SS can be detected. Analysis of *C. burnetii* mutants with a defective Dot/Icm function suggest that the combined activities of the effectors translocated into host cells by the Dot/Icm system are essential for remodeling of the CCV to form a spacious and fusogenic vacuole that permits *C. burnetii* replication and for blocking innate defenses of the host, such as apoptosis. Thus, there are several *Coxiella*-dependent features of the vacuole that require effector translocation by the Dot/Icm system.

block an as-yet-undefined antimicrobial function of the lysosome or promote a new lysosomal activity that creates an environment conducive to replication. Another explanation for how the *L. amazonensis* vacuole allows Dot/Icm-independent *C. burnetii* replication is that it may provide specific nutritional requirements required for growth. Both organisms have several amino acid auxotrophies, which indicates that they likely modulate the intracellular environment to salvage essential metabolites (27, 28). For both organisms, it is presumed that the replicative vacuole is a nutritionally complex compartment containing a range of carbon sources, particularly amino acids, acquired by the continual fusion of the vacuole with a variety of organelles in the secretory and endolysosomal systems. These data may present an opportunity to

extrapolate the finding that Dot/Icm effector proteins that act to facilitate the fusogenicity of the CCV also enable *C. burnetii* replication by providing essential nutrients. In addition, understanding the shared metabolic requirements of *C. burnetii* and *L. amazonensis* may help define the minimal requirements required for replication of each organism.

The advent of axenic culture and genetic tools for *C. burnetii* investigations has opened to an array of new possibilities and increased the understanding of the researchers in the field of how this intracellular pathogen interacts with its host. The confirmation that the Dot/Icm system plays an integral role in this host-pathogen interaction now paves the way for additional specialized studies of the functions of individual effector proteins in modulating the intracellular environment.

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