REVIEW ARTICLE

# Modulation of host cell signaling pathways in response to *Coxiella burnetii* infection

Melissa N. Brewer*, Theresa J. Rayburn, Paris R. Al-khateib, R. Bryce Ferguson, Benjamin E. Lind, Aaron J. Pankey, Kincade S. Hise, Shandra F. Jones, Ning Wu

Department of Biological Sciences, Southeastern Oklahoma State University, Durant, OK, USA.

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***Coxiella burnetii* is an obligate intracellular bacterium that lives in a modified lysosome termed the *Coxiella* containing vacuole (CCV). *C. burnetii* is the causative agent of the zoonotic illness Q Fever, which primarily infects ruminant livestock and can spread to humans *via* inhalation. Acute Q fever is characterized by a flu like illness, while chronic disease is associated with more severe symptoms including endocarditis and chronic fatigue syndrome. *C. burnetii* has a biphasic life cycle consisting of a small cell variant (SCV) and large cell variant (LCV). The SCV is environmentally stable and can cause infection *via* inhalation of 1 - 10 infectious particles. Once taken up by the host cell, *C. burnetii* must manipulate the signaling pathways of the host cell to form the CCV where it switches to the LCV, the metabolic and replicative form of the bacterium. It primarily accomplishes this goal by utilizing a Type IV B Secretion System (T4BSS), which is unique to *C. burnetii* and *Legionella pneumophila*. The T4BSS, along with some other secretion mechanisms, secretes effector proteins into the host cell. These proteins then interfere with or modulate the host cell to recruit vacuoles, evade detection by the immune system, and prevent the host cell from initiating apoptosis. After about 6 days, the LCV will convert to SCV and then initiate host cell lysis to spread infection. This review looked at many eukaryotic cells signaling pathways and the interactions between *C. burnetii* and host proteins. These interactions are responsible for the modulation of host cell pathways necessary for CCV formation and *C. burnetii* survival. Understanding these interactions better will help with future treatments for *C. burnetii* infection. Further discoveries in these interactions are crucial for the future of *C. burnetii* research.**

**Keywords:** *Coxiella*; cell signaling; apoptosis; autophagy.

***Corresponding author:** Melissa N. Brewer, Department of Biological Sciences, Southeastern Oklahoma State University, Durant 74701, OK, USA. Email: mbrewer@se.edu.

## Introduction

*Coxiella burnetii* is an obligate intracellular bacterium and the causative agent of Query (Q) fever, a zoonotic illness. *C. burnetii* has a biphasic life cycle and persists in the environment in its

non-replicative form, known as the small cell variant (SCV), and the replicative form, known as the large cell variant (LCV) that requires a unique niche environment as it only replicates within a modified autolysosome termed the *Coxiella* containing vacuole (CCV). The entry into the host cell requires cytoskeletal rearrangement *via* RhoA and has been confirmed by negative mutation and/or silencing functional proteins of Rho GTPase dominant [1]. The manipulation of the actin cytoskeleton may be an important part of the cell internalization process demonstrated during *C. burnetii* infection [2]. Once *C. burnetii* enters the host cell *via* phagocytosis, the phagosome containing *C. burnetii* follows the traditional endocytic pathway by fusing with a lysosome. The enzyme mTORC1 is a central kinase that governs lysosome biosynthesis, cargo trafficking, and fusion and is linked to bacterial replication [3]. The acidic environment of the phagolysosome is necessary to initiate *C. burnetii* metabolism, which triggers the phase transition of *C. burnetii* from the SCV to the replicative LCV. When *C. burnetii* enters the host cell and begins the formation of the CCV, it converts from the SCV to the LCV form, activates the Type IVB Secretion System (T4BSS) machinery, and initiates the translocation of approximately 130 bacterial effector proteins into the host cell to modulate host cell activity (Figure 1). The T4BSS is unique to *C. burnetii* and *Legionella pneumophilia*. It is responsible for sending bacterial protein effectors into the host cell, modulating host cell signaling pathways, and manipulating the host cell to meet the needs of the CCV. *C. burnetii* has been found to inhibit mTORC1 activity in a T4BSS-dependent manner. The collective actions of the effector proteins secreted by the T4BSS are to create a specialized niche supportive of replication and biogenesis of a mature CCV [4, 5]. The effectors of the T4BSS interact with host cell proteins to modulate host cell gene expression, avoid the host immune response, prevent apoptosis, and direct the development of the CCV by controlling vesicle formation and trafficking [6], which manipulates the host cell to create the niche environment necessary for bacterial replication.

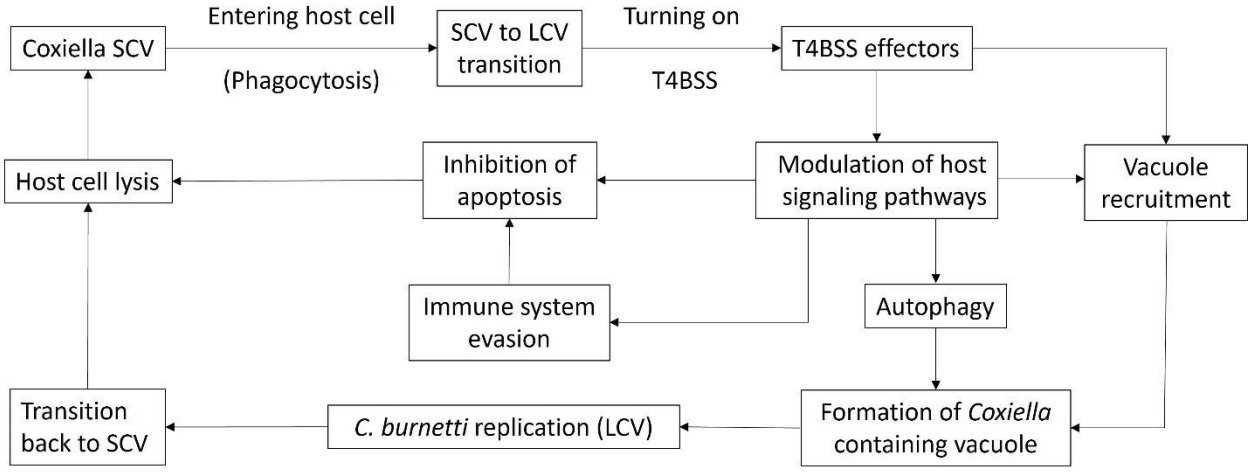


**Figure 1.** The life cycle of *C. burnetii* and the host cell pathways necessary for *C. burnetii* replication and survival. *C. burnetii* replicates withing a niche environment termed the *Coxiella* containing vacuole (CCV).

**Formation of the Coxiella containing vacuole**

**Table 1.** Host and *C. burnetii* proteins involved in CCV formation.

| | Function | The factors in host cell | The factors in *C. burnetii* | Ref. |
|---|---|---|---|---|
| Manipulation of the endocytic pathway | Endocytosis | Protein tyrosine kinase (PTK) | | 52 |
| | Endocytosis | Hck and Lyn | | 52 |
| | CCV homotypic fusion | Rab5 and Rab 7 | Cig2 | |
| | Host clathrin adaptor facilitates interaction with CCV and recycling vesicles | AP-2 | CvpA | 32 |
| | Homotypic fusion | Pi3P and phosphatidylserine | CvpB | 54 |
| | CCV formation | Unfolded protein response (UPR) | | 3 |
| | | Fam 21 | | 10 |
| Cytoskeleton rearrangement | | WASH | | 10 |
| | Actin Polymerization | Arp2/3 | | 10 |
| | GTPase Activity | RhoA | CirA | 36, 56 |
| | Inhibit RIG-1 signaling | Type I interferons | EmcA and EmcB | 29 |
| Vesicle fusion | Phosphorylation of Vasodilator-stimulated phosphoprotein (VASP) | Protein Kinase A (PKA) | | 26 |
| | Activates PKA and EPAC for development of large CCV | Cyclic AMP (cAMP) | | 57 |
| | Lysosome biosynthesis and fusion; Cargo Trafficking | mTORC1 | | 2, 53 |
| | Homotypic fusion | SNARE and Vamp7 | | 57 |

**(CCV)**

**Manipulation of the endocytic pathway** Vacuole recruitment and endocytic pathway manipulation are necessary parts of *C. burnetii* infection of a host cell (Table 1). This manipulation provides necessary nutrients and additional membrane for the growth of the CCV. Protein tyrosine kinase (PTK) is activated during the endocytosis of virulent *C. burnetii* in THP-1 monocytes. Virulent *C. burnetii* showed highly increased PTK activity at the beginning of the infection and then reached baseline levels. Two Src-related proteins (Hck and Lyn) were identified to have high phosphorylation activity and *C. burnetii* activity during *C. burnetii* endocytosis was also highly colocalized in the regions with FActin in THP-1 monocytes infected with virulent *C. burnetii*. However, when PTK inhibitors were introduced, there was very little colocalization of PTK to the cytoskeleton [7].

Upon intake, *C. burnetii* resides in an endocytic vacuole that is transported to lysosomes by the host endocytic machinery and Rab proteins (Rab5

and Rab7). The effector protein Cig2 promoted robust fusion of autophagosomes with the CCV, which is essential for CCV homotypic fusion. Furthermore, the effector protein CvpA interacts with the host clathrin adaptor AP-2 to facilitate interaction between the CCV and endocytic recycling vesicles. Cholesterol enrichment of the CCV is facilitated by the fusion of cholesterol-rich organelles with the CCV. However, the specific effectors that modulate cholesterol have not yet been identified [8]. The CCV in host cells is an important site for *C. burnetii* replication. The CCV is shaped by *C. burnetii* interacting with or manipulating the host cell's endolysosomal and autophagic pathways. The CCV of *C. burnetii* is unique even among intracellular bacteria and is an interesting area of study for pathogen-host interactions [9]. *C. burnetii* manipulates metabolism by secretion of the effector protein, *Coxiella* vacuolar protein B (CvpB) that binds to PI3P and phosphatidylserine on the CCV. CvpB association with the early endosome triggers the clustering of vacuoles for CCV expansion. It favors the association of the autophagosomal machinery to the CCV for optimal homotypic fusion of the *Coxiella*-containing compartments [10]. The unfolded protein response (UPR) is also involved in the formation of the CCV following infection of macrophage cells by *C. burnetii* through its distinct components, such as activating eukaryotic initiation factor 2 (eIF2) downstream of protein kinase RNA-like endoplasmic reticulum kinase (PERK). Previous studies confirmed inhibiting eIF2 activity limited *C. burnetii* growth [11].

**Cytoskeleton rearrangement for CCV formation**

The cytoskeleton plays an important role in the arrangement of organelles and movement of proteins withing the cytoplasm of the cell (Table 1). As such, it is important during *C. burnetii* infection for the cytoskeleton to be rearranged to allow for growth and trafficking of nutrients to the CCV. A study found that actin patches formed on the CCV membrane of THP-1 macrophages. These patches acted as docking sites for vesicle fusion, suggesting they likely played a role in CCV maturation. Formation of these patches relied on the retromer complex, which included Fam21 WASH and Arp2/3. Arp2/3-dependent actin polymerization played a role in CCV formation. Inhibiting Arp2/3 led to vacuole collapse, which disturbed the formation of the CCV. *C. burnetii* protein synthesis was found to be necessary for these processes to occur, implicating bacterial effectors in the role of modifying host cell processes for vacuole formation [12]. Generation of the CCV requires a functional T4BSS to deliver effectors into the host cell. The T4BSS effector CirA stimulates the GTPase activity of RhoA. When CirA is overexpressed, it results in cell rounding and the disruption of stress fibers due to RhoA activation. CirA is the first Rho GTPase interacting effector molecule identified that directly recruits Rho GTPase to promote biogenesis of the bacterial vacuole. CirA is important for coopting host Rho GTPases needed for *C. burnetii* infection establishment and virulence [13]. The T4BSS effector proteins, EmcA and EmcB, are required to inhibit RIG-1 signaling during *C. burnetii* infection. It was found that Type I interferons were detrimental to *C. burnetii* infection, and *C. burnetii* blocks type I interferon production mediated by retinoic acid-inducible gene I (RIG-I) signaling. EmcB is identified as a ubiquitinspecific cysteine protease capable of blocking RIG-I signaling by deconjugating ubiquitin chains necessary for signaling. Additionally, EmcA and EmcB were necessary for inhibiting RIG-I signaling by *C. burnetii*. EmcB was shown to efficiently cleave K63-linked ubiquitin chains, the known activating linkage for RIG-I, and this mechanism likely evolved to enhance infection and persistence in mammalian hosts. Research suggested that *C. burnetii* might have evolved multiple mechanisms to interfere with Type I IFNs during infection [14].

**Vesicle fusion is required for the development of a mature CCV**

Recruitment and fusion of vesicles is important for the formation of the CCV. This allows for the recruitment of nutrients and larger vesicle formation (Table 1). *C. burnetii* has been shown to manipulate vasodilator-stimulated phosphoprotein (VASP) during the progression of vacuole formation. *C. burnetii* actively targets host PKA signaling to phosphorylate VASP, which is essential for the actin-dependent expansion of the CCV. Phosphorylation of VASP at specific residues (S157 and S239) is significant in regulating actin polymerization dynamics and CCV formation. The manipulation of VASP by *C. burnetii* depends on bacterial protein synthesis and an active T4BSS. The activity of VASP is crucial for optimal *C. burnetii* growth and CCV formation in macrophages [15]. Cyclic AMP (cAMP) is an important second messenger for many important roles, including PKA activity and activation of the exchange protein EPAC, a guanine nucleotide exchange factor (GEF) of the GTPase Rap2. EPAC and its downstream effector Rap2b are recruited to the CCV. When Rap2b is overexpressed, it inhibits the development of a large CCV. It inhibits the fusion of early phagosomes, which indicates that the homotypic fusion events are altered when there are high levels of Rap2b. CCV fusion is also altered when GTPase is overexpressed. v-SNARE and Vamp7 are found in cells overexpressing Rap2b, suggesting they also play a role in the fusion events of the CCV [16].

**Autophagy**

Autophagy plays a role in regulating vacuole size and may result in or facilitate resealing of intracellular damaged membranes [20]. As the CCV develops, it resembles an autophagolysosome, marked by autophagy and endolysosomal markers. This resemblance is due to the CCV maintaining conditions like an autolysosome, which include an acidic pH of approximately 4.8 and some notable acid hydrolase activity. Moreover, reactive oxygen

**Table 2.** Host and *C. burnetii* proteins involved in host cell autophagy.

| Function | The factors in host cell | The factors in *C. burnetii* | Ref |
|---|---|---|---|
| LC3B recruitment | Clathrin Heavy Chain (CLTC) | | 46 |
| Vacuole biogenesis and bacterial replication | RAB26 | CvpF | 5 |
| Homotypic fusion | LC3B | | 69, 70 |
| Increase proteins associated with lysosome biogenesis | TFE3 & TFEB | T4BSS Effectors | 6, 25 |
| Stabilizes autophagic machinery | | CvpB/Cig2 | 2 |
| Autophagy and activation of Nrf-Keap1 | p62 | | 13 |
| Oxidative stress protection | Nrf-Keap1 (Nrf-2) | | 13 |
| Replication & CCV expansion | carA, metK, cvpD, and ahcY (mRNA targets) and CsrA2 | CbsR12 (noncoding RNA) | 66 |
| Endosomal trafficking and autophagy | TBC1D5 | CstK | 30 |
| Restrict Cb replication *via* vesicle fusion interference | STX11/Syntaxin and STX8 | | 35 |
| Restrict CB replication *via* tryptophan depletion | IFNy with IDO1 | Silencing RNAs | 37 |
| Autophagic markers | EEA1 and LAMP1 | | 48 |
| Induces apoptosis | Cytochrome C | | 60 |

species (ROS), produced by infected macrophages, are known to inhibit the growth of *C. burnetii* [17]. While many intracellular pathogens stop phagosomes from maturing early on, *C. burnetii* allows for accumulating lysosomal enzymes in their CCVs. This process is more delayed compared to standard phagosomes, and this may be because of the CCV's interaction with the autophagy pathway, which promotes *C. burnetii* replication. Furthermore, the translocation of *C. burnetii*'s T4BSS effectors occurs significantly later after infection, highlighting the idea of different unidentified bacterial proteins interacting with the autophagy pathway [18]. While the role of autophagy in *C. burnetii* infection isn't entirely clear, research has shown that inducing autophagy in host cells before infection increases the load of bacteria, as well as the CCV's size, thus suggesting that autophagy might provide resources for the growth of *C. burnetii*, as well as the transition from small cell variants to significant cell variants, which happens at the same time as the arrest of CCV maturation [19]. The cells of mammals rely on many different genes for a functional autophagy system with many potential targets for *C. burnetii* to manipulate (Table 2). However, an siRNA screen that was designed to identify these targets did not show a significant number of essential genes for host cell autophagy, which is required for *C. burnetii* intracellular replication [21]. The lack of identified *C. burnetii* genes that are essential for autophagy raise questions about the baseline need of the host's autophagy system

for the maturation of the CCV and for *C. burnetii* replication. Current research findings elucidate that the association of autophagy proteins with CCV may not be super significant to the survival and growth of *C. burnetii* [22]. When using an autophagy inhibitor (phosphatidylinositol 3kinase inhibitor 3-methyladenine) to treat HeLa cells and then infecting with *C. burnetii*, the cells treated with the inhibitor showed higher amounts of cells containing more than one CCV. Inhibitor-treated cells had around 80% of cells containing multiple CCVs, whereas the untreated only had 7%, which indicated that inhibiting autophagy made these cells unable to fuse their vesicles for large CCV formation. When the clathrin heavy chain (CLTC) was silenced, there were also multiple vacuoles in *C. burnetii* infected cells. Those research results showed that CLTC was used for making one large CCV and for bringing LC3B to the vesicle. Starved cells also resulted in larger CCVs than non-starved cells, further implicating the role of autophagy in CCV formation [23].

CvpF is an effector protein important for vacuole biogenesis and bacterial replication in epithelial and myeloid cells. CvpF diverts RAB26 dependent autophagy, which promotes CCV biogenesis. A CVP Tn mutant generates CCVs that lack the autosomal marker LC3B, which implicates CvpF in vacuole formation *via* manipulation of autophagy. CvpF potentially drives the transport and maturation of vesicles toward an autolysosomal state. CvpF also interacts with RAB26. The recruitment of Rab26 is necessary for CCV biogenesis. The depletion of Rab26 is correlated with lower CCV size and less LC3B recruitment. CvpF may be acting as a Guanine exchange factor for Rab26. CvpF has been found to stimulate autophagy, and other effectors like CvpB/Cig2 recruit LC3B to CCVs in order to maintain the autolysosomal state [24]. LC3 is usually associated with autophagic vesicles, and it gets broken down when the vesicles merge with either late endosomes or lysosomes. In regular cells, LC3 is found on the membrane of *Coxiella*-containing vacuoles (aka CCVs). However, in cells that do not contain/contain less of GNPTAB (a HeLa cell line that lacks lysosomal hydrolases in their endocytic compartments), there seems to be an accumulation of the LC3 protein inside of the CCVs, instead of it being on their membranes [25]. To figure out whether the buildup of the LC3 was due to increased autophagic activity, the researchers introduced various conditions that would typically boost autophagy such as amino acid starvation. Interestingly, even with the induced autophagy, there wasn't an increase in LC3 within the CCVs of the normal cells. However, researchers have also tried treating cells with bafilomycin A1, a drug that inhibits vacuole acidification and blocks hydrolase activity, and this led to an accumulation of both LC3 and CD63, which is just another protein involved in cell processing [26]. These observations highlighted that, in normal cells, active hydrolases were effectively able to degrade LC3 proteins that were linked to the CCVs, even when autophagy was increased. However, when hydrolase activity was missing, like the researchers observed in the GNPTABdeficient cells, LC3 accumulated inside the CCVs. Furthermore, while some thought that *C. burnetii* might obtain nutrients from the host *via* CCV fusion with autophagosomes, the researchers found that inducing autophagy through methods like amino acid starvation or Torin-1 treatment did not actually benefit *C. burnetii* growth. On the contrary, *C. burnetii* infection did not increase the autophagic flux in host cells [27], which suggested that lysosomal hydrolases might be important for the degradation of autophagic components within the CCVs [28]. A significant increase in the

lysosomal proteins TFEB and TFE3 CLEAR regulatory network has been identified during *C. burnetii* infection. *C. burnetii* induces the nuclear translocation of TFEB and TFE3, promoting an increased abundance of proteins associated with lysosome biogenesis. It has also been found that the sustained nuclear localization of TBEF and TFE3 during *C. burnetii* infection depends on bacterial protein synthesis and the activity of the T4BSS. The research suggested that TFEB and TFE3 were important for of the spacious CCV. However, they did not significantly impact *C. burnetii* replication. The study also revealed a nonredundant role for TFEB and TFE3 in CCV expansion [29]. In autophagyinducing conditions, the size and fusogenicity of the CCV are increased, which implies interplay between *C. burnetii* and host cell autophagy. Activation of TFE3 is linked to increased CCV size [3].

The T4BSS effector protein CvpB/Cig2 stabilizes the autophagic machinery, which is necessary to form a mature CCV [4], while the T4BSS effector protein CvpA has domains for interaction with clathrin. In bacterial strains with Cya and CvpA, there was a 50-fold increase in cAMP in host cells compared to strains with only CyaA or dota mutant. CvpA depends on the T4BSS for translocation into the host cell cytosol. Mutant CvpA *C. burnetii* strains demonstrated a 100-fold decrease in growth compared to wild type, showing the importance of CvpA for replication within the CCV. The research also showed that clathirin did not localize to the CCV in the CvpA mutant. The interaction of CvpA with clathrin is necessary for the growth of the CCV in a T4BSS dependent manner [30]. In a study using *C. burnetii* p62 infected human alveolar macrophages, the results suggested that pathogenicity was not required for such localization. The role of p62 was typically to aid in the selection of targets for autophagy degradation and was degraded itself in the process. But, when cells were infected with *C. burnetii*, p62 did not get degraded, even during a starvation response, which suggested that *C. burnetii* was playing a role in stabilizing p62 and preventing its usual degradation. Additionally, the levels of phosphorylated p62 were shown to increase during infection, indicating that *C. burnetii* played a role in activating p62. *C. burnetii* growth was not found to be dependent on the presence of p62 but it did appear to play a role in signaling. The research identified the activation of the "Nrf-Keap1" pathway during infection, which was indicated by increased Nrf2 levels and movement into the cell's nucleus. The typical role of this pathway was protecting cells against oxidative stress. These results indicated that, while *C. burnetii* infection was not creating a traditional autophagic response, it did alter p62 levels for signaling and host cell manipulation [31]. During *C. burnetii* infection, the small noncoding RNA CbsR12 is highly transcribed and plays a crucial role in regulating various aspects of the infection process such as the replication of *C. burnetii* and the expansion of the CCV within host cells. Studies showed that CbsR12 bound to specific mRNA targets, including carA, metK, cvpD, and ahcY, and regulated their expression. CbsR12 also interacted with the regulatory protein CsrA-2, indicating its dual role as both a transacting sRNA and a CsrA-sequestering sRNA [32]. Signal transduction is a crucial function that allows cells to respond to environmental signals and adapt. The serine/threonine kinases CstK is translocated by *C. burnetii* during infection, where it localizes at the CCV. CstK undergoes autophosphorylation on threonine and tyrosine residues and displays kinase activity towards eukaryotic protein kinase substrates. CstK exhibits features corresponding to secreted effector

proteins, prompting its role as a *Coxiella* effector protein. CstK also interacts with the host protein TBC1D5 which is involved in endosomal trafficking and autophagy. CstK may subvert TBC1D5's function to support efficient *C. burnetii* replication [33]. The involvement of Syntaxin in host cell mediated in disruption of *C. burnetii* replication within the host cell was found through the utilization of two main mechanisms. The host cells stimulated by gamma interferon were able to restrict *C. burnetii* replication through the means of IDO1 depletion of tryptophan, and only at this time, it was through the upregulation and utilization of host protein Syntaxin 11 (STX11). Additional tests showed that, when placing other host cells with altered levels of GFP-tagged STX11, it localized to the CCV. Furthermore, it was found that STX11 in some associated with STX8 was able to modulate and restrict replication of intracellular pathogens, such as *C. burnetii*, *via* interference of vesicle fusion pathways in lysosomal-derived organelles [34]. IFNγ in association with IDO1, identified as an outlying effector among other components of the IFNγ signaling process, could restrict *C. burnetii* replication within the CCV, which was found using silencing RNAs to alter the IFNγ signaling through which IDO1 was found to be essential in the process of restricting *C. burnetii* replication. IDO1 compound catabolized tryptophan, making it less available within the cell. Furthermore, cell deficient in IDO1 and subjected to IFNγ signaling were less effective at restricting *C. burnetii* infection, while in conjunction, if these cells were given excess tryptophan, *C. burnetii* replication was more successful. In direct correlation ectopic expression of IDO1 in the absence of IFNγ signaling resulted in *C. burnetii* restriction. All those research results suggested that IFNγ signaling activation of IDO1 was an essential host mechanism for restricting *C. burnetii* replication. Additionally, tryptophan was needed for bacterial replication and inadequate tryptophan derived from hydrolysis of proteins in the CCV affected bacterial replication [33]. Cells infected with *C. burnetii* begin secreting T4BSS effector proteins after the cell endocytoses the bacteria. Past study confirmed that dot/icm mutant *C. burnetii* strain infected HeLa cells did not show translocation of tagged effector proteins no matter the MOI, indicating the importance of the T4BSS system in translocating these proteins into the host cytosol. Looking at the acidity of the host vacuole, when it was inhibited by Bafilomycin A, an inhibitor of vacuolar ATPase, there was no detectable translocation of these tagged proteins even 24 hours after infection, indicating the importance of acidity in translocation. Endocytic maturation was also found to be important for the translocation of effector proteins by the T4BSS. In infected macrophages, translocation was detected after 4 hours [34]. The acidic environment of the CCV is necessary for *C. burnetii* replication. The T4BSS has been shown to play a role in regulating the maturation and, as a result, the acidity of the CCV. Endosomal markers EEA1 and LAMP1 have been measured to determine the role of *C. burnetii* in the maturation in the delay of maturation endosomes. Wild-type results in a greater reduction of LAMP1 compared to mutants, but there is no significant difference in the levels of EEA1. Because autophagosomes fuse with lysosomes to form the autophagolysosome, the autophagy markers LC3 and p62 are overexpressed in *C. burnetii* infected cells. Experimental results indicated that suppressing autophagy might not affect lysosomal content. location. The location also affects CCV acidity as it migrates through the cell. They are less acidic near the cell periphery but become more acidic as they migrate toward the perinuclear area toward the center of the cell. When wild-type cells were

compared to T4BSS mutant cells, they showed less average acidity of the CCV in the perinuclear area, indicating that the T4BSS might be responsible for controlling acidity in the CCV maturation process. The T4BSS mitigates the molecules involved in endosomal maturation to maintain the correct acidity of the CCV for *C. burnetii* growth [35].

**Apoptosis**

*C. burnetii* can manipulate apoptosis in affected host cells. It uses different proteins to either inhibit or induce apoptosis (depending on the cell type). For instance, it can degrade pro-apoptotic proteins, upregulate genes for antiapoptotic proteins, or even fully block the intrinsic apoptotic pathway (Table 3). Cells infected with *C. burnetii* show varying responses to apoptosis, influenced by the cell's type, maturation, and surface receptors [36]. For example, it was found that infection in certain mice cells inhibited apoptosis, but in others, it induced cell death [37]. This response difference could be part of the strategy *C. burnetii* uses to establish a strong infection. *C. burnetii* uses several mechanisms to restrict apoptosis, such as effector protein dissemination through the T4BSS and BCL-2 family proteins, as well as the upregulation of pro-cell survival mechanisms like ERK, Akt, and PKA with the last few components activated at different times throughout infection for different purposes such as cell survival and CCV formation. Those components are important for *C. burnetii* survival and CCV replication/expansion including the use of IRE1 and PERK independent of CaeB proteins. Several effector proteins associated with antiapoptotic pathways were identified, including AnkG, CaeA, and CaeB, which might alter the autophagy regulation and the effectors associated with the host cell change after *C. burnetii* infection [38]. Cells infected by *C. burnetii* use effector proteins secreted by the T4BSS to avoid the activation of apoptosis [39]. One mechanism *C. burnetii* uses to accomplish this is by sequestering the protein Bad away from mitochondria in a PKA-directed manner, which prevents the activation of Cytochrome C and the initiation of the apoptotic pathway [40]. Previous studies demonstrated that when *C. burnetii* infected HeLa cells were exposed to radiation, the cells showed decreased levels of apoptosis. However, after using staurosporine to inhibit the protein kinase, inducing the intrinsic apoptosis pathway, more fragmented DNAs were observed in uninfected cells than in infected cells. Infected cells introduced to staurosporine showed much

**Table 3.** Host and *C. burnetii* proteins involved in regulation of host cell apoptosis.

| Function | The factors in host cell | The factors in *C. burnetii* | Ref |
|---|---|---|---|
| Regulation of apoptosis | BCL-2 Family Proteins | T4BSS Effectors | 43 |
| Cell survival and CCV formation | ERK, Akt, and PKA | AnkG, CaeA, CaeB | 43, 34 |
| ER Stress Response | IRE1 and PERK | CaeB | 43, 14 |
| Sequestered away from mitochondria to prevent apoptosis | Bad | T4BSS Effectors | 58 |
| Maintained at modulated levels | NF-kB Pathway | T4BSS Effectors | 76, 77 |
| Inhibited during infection | p32-mediated apoptosis pathway | AnkG | 8,12, 63 |
| Inhibition of apoptosis | Poly ADP-ribose polymerase | T4BSS Effectors | 9, 11 |
| Inhibition of apoptosis | A1/bfl-1 and c-iap2 | T4BSS Effectors | 11 |
| Changes in cell signaling including apoptosis pathways | E-cadherin/B-catenin Signaling | | 63 |
| Activation of Survival Kinases Akt and Erk1/2 | Protein Tyrosine Kinases | | 1 |
| Antiapoptotic activity | Akt and Erk1/2 | | 1 |
| Inhibition of apoptosis | cJun, Hsp27, JNK, and p38 | | 38 |
| Reduce autophagy and prolong phagosomal maturation; delay apoptosis | Multiple apoptosis regulating pathways | miRNAs including miR-1433p | 41 |
| Disrupts TLR signaling preventing immune detection | Inhibits activation of p38-MAPK | Virulent LPS | 45 |

less caspase 3/7 activity than noninfected cells in the same condition. In addition, infected cells with staurosporine showed a decrease in Cytochrome C compared to uninfected cells in the same condition [41]. The NF-kB pathway is an important pathway for regulating proinflammatory responses and apoptosis. When cells are infected with *C. burnetiid*, RelA (the subunit of NF-kB) showed higher levels of phosphorylation than non-infected cells. When bacterial protein synthesis was inhibited by chloramphenicol, a higher level of RelA activation was observed than that in non-inhibited *C. burnetii*. Those results indicated that *C. burnetii* modulated the levels of RelA activation. The modulation of NF-kB activity also occurred *via* the non-canonical pathway. When the T4BSS was inactivated, RelA activation was like that of *C. burnetii* with no protein production, indicating that the modulation of NF-kB activity by *C. burnetii* was T4BSS dependent. Also, *C. burnetii* affects both the intrinsic and the extrinsic apoptosis pathways, and it inhibits host cell apoptotic processes by preventing the release of Cytochrome C and caspase activation. This may possibly be done by upregulating different proteins and injecting effector proteins into the cell via the T4BSS [42, 43]. Moreover, the specific proteins produced by *C. burnetii* can play roles in

antiapoptotic processes. For example, AnkG inhibits the p32-mediated apoptosis pathway in mammal cells, and this requires nuclear localization to prevent cell death most effectively. Next, CaeB targets the endoplasmic reticulum stress sensor IRE1, and this inhibits ER stress-induced apoptosis [44]. AnkG is a T4BSS effector that has been studied for its role in interfering with apoptosis. Deletion of the 69 amino-terminal amino acids of AnkG results in a lack of the predicted domains crucial for its antiapoptotic activity. This region was found to bind to the mammalian protein p32. In mammalian cells, a silencing of p32 protects against UV-induced apoptosis. The binding of AnkG to p32 shows how AnkG interferes with host cell apoptosis. *L. pneumophila* lacks the antiapoptotic factor seen in *C. burnetii* but gains the ability to inhibit apoptosis when AnkG is introduced [45]. AnkG has been shown to vary among different strains with three different AnkG protein structures. First is the Nine Mile strain (AnkGNM), a full-length protein (338 amino acids, 38.6 kDa) that can inhibit apoptosis. The second group, which has thirteen “isolates” with a deletion of two base pairs after the 83$^{rd}$ amino acid, results in a shorter 92 amino acid protein (called AnkGSoyta) with a mass of 10.4 kDa. Although the first 83 amino acids of AnkGSoyta are identical to the Nine Mile strain, the rest of the sequence varies quite a bit in size and structure. The third group with four isolates is called AnkGF3, and it is unique from the others due to an amino acid mutation (isoleucine to leucine at position 11). This mutation is also found in the “Dugway strain,” and it slightly aids the protein's ability to protect cells from “staurosporine-induced apoptosis” [46]. Also, a base pair insertion at this group's 29$^{th}$ amino acid codon leads to a premature stop at codon 51, creating a smaller AnkG variant, AnkGF3, of 6 kDa. This variant shares the same sequence as AnkGNM in the first 28 amino acids, but the 23 amino acids following that do not. The third type showed an increased resistance to staurosporine-induced apoptosis. Although they have various structural differences, all the AnkG variants showed the ability to bind to host proteins that are used in nuclear localization (necessary for them to function). However, their effectiveness in inhibiting or promoting apoptosis is varied. AnkGNM inhibited apoptosis, AnkGSoyta did not have any effect on it, and ANKGF3 promoted apoptosis. The researchers think that these differences may be because of their structural alterations. To determine whether the protein's length contributed to the variant’s lack of antiapoptotic activity, a truncated AnkGNM 1–92 variant was created that had the same length as AnkGSoyta. The truncated AnkGNM variant was still effective in inhibiting staurosporine-induced apoptosis, so this indicated that length was not a key factor in AnkGSoyta's lack of antiapoptotic activity [47]. This also indicates that the specific amino acids 84–92 in AnkGSoyta as potentially influence the protein's folding, or perhaps its intra/intermolecular binding ability, which in turn affects its functional role in the apoptosis pathway [46]. *C. burnetii* NMI induces apoptosis in a dose-dependent manner during early infection. This antiapoptotic process has been confirmed by analyzing morphological changes, DNA fragmentation, and cleavage of Poly ADPribose polymerase (PARP). During NMII infection PARP is activated but caspase-3 is not. This indicates that the inhibition of apoptosis happens via a caspase-3-independent pathway [48]. Apoptosis has been shown to be inhibited in both THP-1 cells and primary macrophages infected with *C. burnetii*. This resistance is related to reduced cleaved caspase-3, caspase-9, and PARP. Additionally, *C. burnetii* infection affects the expression of genes that are both pro- and antiapoptotic. These

interactions include upregulation of A1/bfl-1 and c-iap2. The antiapoptotic response of *C. burnetii* requires protein synthesis as well as RNA. The antiapoptotic activity of *C. burnetii* is highly correlated with its development cycle and possibly also in pathogen dissemination during an immune response [49]. *C. burnetii* has a significant effect on the E-cadherin/β-catenin signaling pathway during infection, seen when *C. burnetii* alters the expression of E-cadherin on the surface of the host cell. The interaction of *C. burnetii* with host cells causes different changes in cell signaling, and this is especially true for the apoptosis pathway [47]. Regarding this, researchers have noted the activation of protein tyrosine kinases by *C. burnetii* in human macrophages, as well as the continued activation of the survival kinases “Akt and Erk1/2”, which are necessary for the antiapoptotic activity [6]. The researchers also saw a pattern in patient samples that were infected with *C. burnetii* where there was an overexpression of genes that are associated with antiapoptotic processes and a simultaneous repression of genes that promote apoptosis [50].

*C. burnetii* infection causes transient phosphorylation of cJun, Hsp27, JNK, and p38 and sustained activation of Akt and Erk1/2 pathways. Along with these results, it was determined that Akt and Erk1/2 activation are required for *C. burnetii* inhibition of host cell death [51]. *C. burnetii* limits host cell apoptosis, lengthening *C. burnetii* infection and establishing reproductive environments and a positive environmental niche within the cell. The *C. burnetii* effector protein CaeA accumulated in the host cell nucleus, altering the normal apoptosis pathways. A positive correlation in CaeA antiapoptotic effects has been found in previous research, and CaeA hindered cleavage of caspase 7 in the associated apoptotic pathways, but not caspase 9 has also been confirmed. Inhibitors of apoptosis (IAPs) such as the protein survivin are known to inhibit caspase 7 cleavage. These specific IAPs were upregulated in the presence of CaeA protein, which indicated its involvement in antiapoptotic mechanisms. Several other aspects were also investigated, including the requirement of at least four EK repetitive motifs in the CaeA protein to enable apoptosis hindrance and EK deficient CaeA mutants that could not inhibit apoptosis of the host cell [52]. Micro RNAs' roles in normal cellular apoptosis and autophagy are associated with the cascades of cellular processes that occur during pathogenic infection. This can be witnessed in the role of miR-143-3p specifically. However, micro RNAs such as miR-125a-3p can have the inverse effect of reducing autophagy and prolonging phagosomal maturation, favoring intracellular pathogen survival. A past study found more than 80 miRNAs that were in relation to the apoptotic pathway during *C. burnetii* infection and an established timeline for upregulation and downregulation peaks of miRNA expression. The infection differentials associated with the LPS coat, and the expression of genes associated with delayed apoptosis, such as akt1 and bcl2. The results suggested that miRNA was directly involved in the apoptotic pathway during *C. burnetii* infection [53]. Virulent LPS induces cytoskeleton rearrangement in macrophages, which inhibits the activation of p38a-MAPK and disrupts the association of TLR-2 and TLR-4. This interference with TLR signaling allows *C. burnetii* to evade detection and enhance its survival in macrophages.

Reorganization of the cytoskeleton by LPS also plays a crucial role in hindering TLR-2 and TLR-4 association and p38a-MAPK activation. Because cytochalasin D inhibits cytoskeletal rearrangement, it can also be used in the recovery

of p38a-MAPK activation and the coimmunoprecipitation of TLR-2 and TLR-4 [54]. When measuring p38 that activates MAPK and leads to cytokine production in infected MyD88deficient Bone Marrow Derived Macrophages (BMDMs), p38 was found to be low, which indicated that p38 activation was mediated through the TLR pathway. Type 1 IFN was found to have no role in mitigating *C. burnetii* replication. When knocking out FNF in the BMDMs and infecting them, robust replication in the mutants was observed, indicating a role for TNF in restricting *C. burnetii* growth. Adding TNF to the TLR2 knockouts would also restrict *C. burnetii* growth, which indicated it to be downstream of TLR2. When MyD88 or Trif is knocked out, the cells produce a significantly lower, but still detectable, level of cytokines following *C. burnetii* infection. The double knockout mutants, however, produced undetectable levels of cytokines. The knockouts were all permissive to CCV growth, but the Trif mutants showed the least growth, and the double knockouts showed the most, implicating these adaptor's roles in mitigating *C. burnetii* replication [55]. The TLR and cytosolic innate immune pathways were studied to determine if either can detect or restrict *C. burnetii.* In TLR2 and TLR4 knockouts following *C. burnetii* infection, the cytokines were significantly reduced when TLR2 was knocked out, but not in the TLR4 knockout, indicating TLR2 has a larger role in activating downstream cytokines. When TLR2 is knocked out, more *C. burnetii* growth occurs than in the TLR4 knockout mutants. Double knockouts were also tested with growth similar to the TLR2 knockout mutants [55].

**Host cell immune defense**

*C. burnetii* also uses many methods to help itself avoid the host cell's immune defenses, such as

**Table 4.** Host and *C. burnetii* proteins involved in host cell immune response.

| Function | The factors in host cell | The factors in *C. burnetii* | Ref |
|---|---|---|---|
| Initiates Autophagy | BECN1 | | 78 |
| Antiapoptotic | BLC2, ERK1 (MAPK3), ERK2 (MAPK1) | | 78 |
| Changes in Cell Signaling | E-cadherin | | 78 |
| Inflammatory Cytokines | IL-6, TNFa, and IL-1B | | 44 |
| Anti-inflammatory Cytokine | IL-10 | | 44 |
| Stabilization and increased production of Mcl-1 | MAPK, Erk, and P13K | T4BSS Effectors | 39 |
| Antiapoptotic | Mcl-1 | T4BSS Effectors | 39 |
| Immune response and MAPK Pathway | IL-17 | T4BSS Effectors | 2, 79 |
| Immune Response and Autophagy/Apoptosis | NF-kB Pathway | CinF | 2 |
| Induce Expression of IRG1 | Tumor Necrosis Factor (TNF) and Type I Interferon (IFN) | | 23 |
| Produces an antimicrobial metabolite | IRG1 | | 23 |
| Shifting Cellular Metabolism and Orchestrating Immune Responses, Apoptosis, and Autophagy | Hypoxia-inducible factor-1 (HIF1a and HIF1b) | T4BSS Effectors | 24 |
| Plasmacytoid Dendritic Cell (pDC) activation | TNF and IL-10 | | 42 |
| Proinflammatory Cytokine Production | IL-17 Signaling | T4BSS Effectors | 64 |

resisting apoptosis even after external stimulation with pro-apoptotic drugs (Table 4). Apoptosis is the programmed death of a cell, and it is a key defense mechanism because it removes infected cells and allows for immune responses. The interaction between BECN1 (an autophagy initiation protein) and BCL2 (an antiapoptotic protein) on the CCV prevents Cytochrome C release, and in doing so it inhibits apoptosis. Researchers have also noted another form of antiapoptotic activity seen in the extended activation of survival signaling proteins like ERK1 (aka MAPK3) and ERK2 (MAPK1) [56]. This method of balancing apoptosis inhibition and induction is important for maintaining *C. burnetii* infections and allowing *C. burnetii* to spread to new hosts [19]. *C. burnetii* can transform B cells into a similar state as hairy cells (distinct from those seen in hairy cell leukemia). This transformation causes the reorganization of the cytoskeleton and inhibits apoptosis in host cells. In mouse models, peritoneal B1a cells were noticeably susceptible to *C. burnetii* infection. *C. burnetii* also impacts the expression of Ecadherin in the circulating cells of patients with Q fever. Furthermore, studies have indicated a possible link between *C. burnetii* infection and Bcell non-Hodgkin lymphoma. However, the transformation of B cells into hairy cells doesn't appear to be connected to the BRAF V600E mutation (a mutation typically associated with hairy cell leukemia). This highlights how *C. burnetii* affects the cytoskeleton and apoptosis pathways in the host cells. This may have even bigger implications,

not just for understanding *C. burnetii* infection but also for other medical conditions like myopathies, B-cell disorders, and autoimmune syndromes [57]. Peripheral blood mononuclear cells (PBMCs) of chronic Q fever patients have been used to measure cytokine response to both viable and heat-killed *C. burnetii*. Proinflammatory cytokines, including IL6, TNFa, and IL-1B, were much lower in viable infections than heat-killed ones in naive serum, while IL-10 was much higher in viable infections in naive serum than heat-hilled. There were also differences in cytokine production in chronic Q fever patients compared to naive patients, notably in how IgG specific to *C. burnetii* CXL9 and IFN-y are more upregulated in chronic patients than in naive, but there is not a significant difference between these levels with serum infected with viable or heat-killed bacteria [58]. *C. burnetii* alters and delays apoptosis of neutrophils in the host cell. One theory proposed by the article indicates that *C. burnetii* might use neutrophils as a transportation method to infect macrophages as *C. burnetii* can neither infect neutrophils nor is *C. burnetii* cleared / effectively controlled by neutrophils, which is achieved by *C. burnetii* alteration of neutrophil apoptosis and prolonged life span/cycle. Pathways in this process indicated increased activation of MAPK, Erk, and P13K and the stabilization and production of antiapoptotic proteins such as Mcl1. Furthermore, these observations were evidenced by results such as the introduction of inhibitors to the MAPK and P13K pathways, resulting in a decreased ability to prevent apoptosis. Western blots were the main source of data and analysis of results in this study. One other interesting notation was the evidence of involvement of the T4BSS in the prevention of apoptosis of neutrophils, in that a *C. burnetii* T4BSS mutant was noticeably less able to regulate apoptosis than wild-type *C. burnetii* as a comparison [37].

*C. burnetii* uses T4BSS to insert effector proteins directly into the host cell; these proteins disrupt the activation of the IL-17 gene, which is a critical part of the immune response. This causes a ripple effect that eventually suppresses some of the key proteins used in the MAPK signaling pathway, including NF-κB, MAPK, and JNK [59]. Sometimes, this inhibition can cause oxidative stress triggered by IL-17 to eliminate pathogens [4]. By blocking the oxidative stress response, *C. burnetii* reduces the host's immune cell's effectiveness in fighting an infection, allowing *C. burnetii* to survive and replicate within the host [60]. Moreover, through T4BSS, *C. burnetii* can suppress the activation of the NLRP3 inflammasome. One of the survival strategies regarding apoptosis involves targeting the NF-κB signaling pathway by manipulating the CinF protein (which works to inhibit NF-κB activation). It does this by dephosphorylating IκBα, a key inhibitor in the NF-κB pathway, to stop any immune responses that could lead to the elimination of *C. burnetii* in the host cell. The inhibition of NF-κB activation by CinF causes less expression of immune system cytokines, helping *C. burnetii* to avoid being noticed by the immune system, thus enhancing its survival inside the host cell. Also, CinF might influence the interaction between NF-κB signaling and autophagy, and this could affect the usage of the autophagy marker "LC3" in the CCV [61]. Tumor Necrosis Factor (TNF) and Type I Interferon (IFN) signaling work together to induce the expression of the enzyme IRG1, which in turn produces the antimicrobial metabolite itaconate. Itaconate is shown to restrict *C. burnetii* replication within macrophages, ultimately providing insight into the cell signaling mechanisms underlying the host defense against *C. burnetii* [62].

Hypoxia-inducible factor (HIF)-1, a heterodimer consisting of HIF1a and HIF1b, is crucial for shifting cellular metabolism and orchestrating immune responses. The activity of HIF1 is regulated by proteasomal degradation of the HIF1a subunit, which is controlled by prolyl hydroxylases (PHDs) requiring oxygen, $Fe^{2+}$, and 2-oxoglutarate for HIF1a hydroxylation. A previous study extensively explored the impact of HIF1a stabilization under hypoxic and normoxic conditions, indicating that bacterial, viral, fungal, and parasitic infections could induce HIF1a stabilization. HIF1 was involved in gene transcription, metabolic reprogramming, immune responses, apoptosis, and autophagy. Notably, the research delved into the interaction between macrophages and *C. burnetii*, implicating HIF1a impeding *C. burnetii* replication, bacterial viability, and subsequent persistence. The research findings unveiled that *C. burnetii* could curtail HIF1a stabilization in a manner dependent on bacterial viability, protein synthesis, and the type IV secretion system (T4SS) of the pathogen. Additionally, the research discussed how *C. burnetii* infection under hypoxic conditions led to a pronounced proinflammatory gene expression profile. The data also suggested that *C. burnetii* could influence HIF1a stability and activity, which might affect host-pathogen interactions and disease outcomes [63]. Mast cells (MCs) employ an extracellular microbicidal mechanism to eliminate *C. burnetii*. This is distinct from the internalization and killing of bacteria observed in monocytes. MCs were discovered to release cytonemes, long F-actin extensions enriched with antimicrobial agents, which trap and kill *C. burnetii*. The formation of cytonemes was found to be dependent on the crosstalk between TL-4 and CD36. Cytonemes also kill *C. burnetii*, which is associated with the absence of phagocytosis [64].

Plasmacytoid Dendritic Cells (pDCs) are a subset of dendritic cells that produce type 1 interferon and inflammatory cytokines in response to viral infection. Lymphomas that contain *C. burnetii* have reportedly high pDC levels. *C. burnetii* has been shown to activate pDCs. It also induces higher TNF and IL-10 expression. In patients with chronic Q fever leading to endocarditis, there was a 30% decrease in living pDCs, indicating that acute *C. burnetii* infection led to the increase in pDCs [65]. Inflammatory genes lowered in *C. burnetii* infection are significantly upregulated in infection with T4BSS mutants. One of these is IL17. T4BSS mutants are more sensitive to IL-17, leading to increased expression of downstream signaling genes such as proinflammatory cytokine and chemokine genes. The T4BSS also downregulates macrophage inflammatory protein 2. *C. burnetii* is likely downregulating IL17 signaling using the T4BSS to escape the immune response. In bovine macrophages, the early general upregulation of Th1-related cytokines such as IL-1B, IL-12, and TNF-a in response to the inflammation rapidly declined once *C. burnetii* began replicating. Inhibited translation and release of IL-1B also did not stimulate the expression of activation markers (CD40, CD80, CD86, and MHC molecules) and likely served as a way to help *C. burnetii* evade the immune system [66].

## Conclusion

While many signaling pathways have been found to be modified by *C. burnetii* infection and secreted effectors, much remains to be seen about how *C. burnetii* affects host cell signaling. The functions of many *C. burnetii* effector proteins have not yet been discovered, which may lead to new information on the mechanisms utilized by *C.*

*burnetii* to hijack cell machinery and create the niche environment of the CCV in which it replicates. The T4BSS especially is of interest in future studies on cell signaling affected by *C. burnetii* infection. While several T4BSS effector proteins were mentioned as known modulators of signaling pathways in host cells. The function of many T4BSS effector proteins remains so far unknown, and these additional proteins may serve similar roles in affecting the host cell to optimize it for *C. burnetii* growth. Additional future studies on how *C. burnetii* modulates host cell processes may also help to uncover more information about how these signaling pathways are controlled within the host cell, which may be important for other cellular processes as well. Overall, while there are many host cell modulations that are already well characterized, there remain many interesting areas of study to discover more about both how *C. burnetii* survives within and manipulates the host cell. Having a greater understanding of how host cells interact with *C. burnetii* will help us gain a better understanding of how *C. burnetii* evolved to persist in a hostile niche environment. A better understanding of these interactions will also help to find better vaccines and treatments in the future.

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