Exploitation of host cell biology and evasion of immunity by Francisella tularensis

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Francisella tularensis is an intracellular bacterium that infects humans and many small mammals. During infection, F. tularensis replicates predominantly in macrophages but also proliferate in other cell types. Entry into host cells is mediated by various receptors. Complement-opsonized F. tularensis enters into macrophages by looping phagocytosis. Uptake is mediated in part by Syk, which may activate actin rearrangement in the phagocytic cup resulting in the engulfment of F. tularensis in a lipid raft rich phagosome. Inside the host cells, F. tularensis resides transiently in an acidified late endosome-like compartment before disruption of the phagosomal membrane and escape into the cytosol, where bacterial proliferation occurs. Modulation of phagosome biogenesis and escape into the cytosol is mediated by the Francisella pathogenicity island-encoded type VI-like secretion system. Whilst inside the phagosome, F. tularensis temporarily induce proinflammatory cytokines in PI3K/Akt-dependent manner, which is counteracted by the induction of SHIP that negatively regulates PI3K/Akt activation and promotes bacterial escape into the cytosol. Interestingly, F. tularensis subverts CD4 T cells-mediated killing by inhibiting antigen presentation by activated macrophages through ubiquitin-dependent degradation of MHC II molecules on activated macrophages. In the cytosol, F. tularensis is recognized by the host cell inflammasome, which is down-regulated by F. tularensis that also inhibits caspase-1 and ASC activity. During late stages of intracellular proliferation, caspase-3 is activated but apoptosis is delayed through activation of NF-κB and Ras, which ensures cell viability.

Keywords: tularemia, ASC, caspase, apoptosis, Ras, Akt, SHIP

INFECTION BY FRANCISIELLA TULARENSIS

Tularemia is a zoonotic disease caused by Francisella tularensis, a facultative intracellular pathogen that infects a broad range of small mammals and humans (Ellis et al., 2002; Pechous et al., 2009; Santic et al., 2010a). Four subspecies of F. tularensis have been identified to date (Keim et al., 2007; Nigrovic and Wingerter, 2008; Santic et al., 2009) and they share about 97% genomic identity (Champion et al., 2009; Larsson et al., 2009). These are subspecies tularensis, holarctica, mediastatica, and novicida. Disease in humans is mostly caused by subspecies holarctica and holarctica. Subspecies tularensis is found in North America and is the most virulent, causing the most severe form of tularemia. In contrast subspecies holarctica is distributed throughout the northern hemisphere and causes a mild form of tularemia (Santic et al., 2006). Subspecies novicida does not cause disease in humans but causes a disease in mice that is similar to the disease in humans.

Francisella tularensis is transmitted to humans through inhalation of contaminated aerosol or ingestion of contaminated food and water, a bite by an arthropod vector, or direct contact with infected animals through skin abrasions (Ellis et al., 2002). Clinical presentation of disease depends on the route of infection and include pneumonic tularemia, oropharyngeal tularemia, and glandular or ulceroglandular tularemia (Ellis et al., 2002). Occasionally, F. tularensis can also infect the eye resulting in ocularoglandular tularemia (Harrell and Whitaker, 1985). Ulceroglandular tularemia is characterized by an ulcer at the infected site with swelling of the regional lymph node. Glandular tularemia is similar to ulceroglandular but without the ulcer. In oropharyngeal tularemia the ulcer occurs in the mouth with swelling of the lymph nodes around the neck region. Irrespective of the route of infection the bacteria ultimately enter the blood stream, causing typhoidal tularemia, which leads to septicemia (Oyston et al., 2004; Nigrovic and Wingerter, 2008). Symptoms of the typhoidal tularemia include headache, fever, chills, nausea, diarrhea, and myalgia (Oyston et al., 2004; Nigrovic and Wingerter, 2008). Due to the high morbidity and mortality rate, the ease of dissemination and the fact that inhalation of as few as 10 organisms of subspecies tularensis can cause disease, F. tularensis has been classified by the CDC as a category A select agent.

Once inside the mammalian host, F. tularensis enters and replicates in macrophages (Anthony et al., 1991; Conlan and North, 1992; Fortier et al., 1994). However, there is increasing evidence that the organism can infect other cell types including neutrophils, dendritic cells, hepatocytes, and lung epithelial cells (Pechous et al., 2009). During infection, bacteria migrate from the initial site of infection to the liver and spleen where they replicate (Eigelsbach et al., 1962; Conlan et al., 2003). Although it has been shown that F. tularensis exhibit extracellular phase during in vivo infection in mice (Forestal et al., 2007; Yu et al., 2008), there is no data demonstrating extracellular growth during human or animal infection.

Available data indicate that intracellular trafficking of F. tularensis is similar in macrophages, neutrophils, epithelial cells, and Drosophila melanogaster S2 cells suggesting trafficking might be similar in all cell types (Golovliov et al., 2003a; Clemens et al., 2004; Santic et al., 2005a; McCaffrey and Allen, 2006; Craven et al., 2008; Santic et al., 2009).
*F. tularensis* enters into host cells through binding to surface receptors. This results in the uptake of the bacterium in a spacious loop by a mechanism referred to as looping phagocytosis (Clemens et al., 2005). Uptake by neutrophils and dendritic cells is dependent on opsonization (Lofgren et al., 1983; Ben Nasr et al., 2006) whereas entry into macrophages is through both opsonin dependent and independent mechanisms (Clemens et al., 2005; Balagopal et al., 2006; Pierini, 2006; Schulert and Allen, 2006; Barel et al., 2008). Inside the host cell, the bacteria reside transiently in a phagosome before escaping into the cytosol (Figure 1; Golovliov et al., 2003b; Clemens et al., 2004; Santic et al., 2005a,b; Checroun et al., 2006; Santic et al., 2007; Bonquist et al., 2008; Santic et al., 2008; Qin et al., 2009). Escape is preceded by modification of the phagosome to an acidified late endosome-like compartment (Fortier et al., 1995; Chong et al., 2008; Santic et al., 2008). Within this acidified compartment *F. tularensis* activates virulence genes that allow it to disrupt the phagosome membrane and escape into the cytosol (Chong et al., 2008; Santic et al., 2008).

Once inside the cytosol, the bacteria is recognized by the host cell inflammasome resulting in the cleavage of IL-1 and IL-18 (Figure 1; Mariathasan et al., 2005; Gavriliu et al., 2006; Henry et al., 2007; Fernandes-Alnemri et al., 2010; Jones et al., 2010). Similarly, there is activation of caspase-3 through both the extrinsic and intrinsic pathways between 6 and 12 post-infection. Although caspases are activated early during infection (Lai and Sjostedt, 2003; Mariathasan et al., 2005; Santic et al., 2010b), *F. tularensis* is able to delay death of the cells for its survival and replication by activating NF-κB and Ras both of which stimulate cells survival (Al-Khodor and Abu Kwaik, 2010; Santic et al., 2010b). During late stages of infection of mouse macrophages, *F. tularensis* is taken up in an autophagy-like compartment (Checroun et al., 2006). However, this re-entry of *F. tularensis* into the endosomal–lysosomal pathway through autophagy does not occur in human macrophages, and therefore is not relevant to infection of humans (Akimana et al., 2010). Toward the end of the infectious cycle, the induction of apoptosis allows the bacteria to disrupt the cytoplasmic membrane and escape the spent cell to begin new infectious cycle (Figure 1).

**ENTRY INTO AND REPLICATION WITHIN HOST CELLS**

*Francisella tularensis* enters primary macrophages through both opsonin dependent and independent mechanisms. Complement-opsonized bacteria enter macrophages either through complement receptor 3 (CR3) or the scavenger receptor A (SRA) (Clemens et al., 2005; Pierini, 2006). Antibody-opsonized *F. tularensis* enters macrophages through FC gamma receptor (Balagopal et al., 2006) in contrast to unopsonized bacteria that enter macrophages through binding to the mannose receptor and surface nucleolin (Balagopal et al., 2006; Schulert and Allen, 2006; Barel et al., 2008). It has also been shown that opsonization of *F. tularensis* with lung collectin surfactant protein A (SP-A) enhance bacterial uptake by primary macrophages but the host cell receptor is not known (Balagopal et al., 2006). Similarly, the bacterial ligand for mannose receptor

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**FIGURE 1 | Intracellular trafficking of *Francisella tularensis* within macrophages.** *F. tularensis* enters macrophages using different receptors and resides transiently in the FCP which acquires EE1 followed Lamp-1, Lamp-2, and Rab7 but excludes Cathepsin D. Within 30 min of infection the FCP acquires vATPase enabling *F. tularensis* to acidify the FCP and escape into the cytosol. Within the cytosol, *F. tularensis* activates caspase-1 and caspase-3 but delays pyropoptosis and apoptosis and maintain cell viability till late stages of infection when the bacteria exit the spent cell.
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Intracellular life cycle of Francisella tularensis

In contrast to other intracellular bacteria such as Salmonella typhimurium, which requires PI3K to form the phagocytic cup, the uptake of F. tularensis is not affected by inhibition of the PI3K pathway (Parsa et al., 2006, 2008). This is consistent with a different mechanism used by F. tularensis to enter into host cells (Clemens et al., 2005). In addition to actin microfilament, the entry of F. tularensis into macrophages has been shown to be dependent on cholesterol-rich lipid domains known as lipid rafts since lipid rafts-associated components such as cholesterol and caveolin-1 are incorporated into the Francisella-containing phagosome (FCP) membrane upon its biogenesis from the macrophage plasma membrane (Tamilselvam and Daehler, 2008). The recruitment of lipid rafts to the FCP may act as a platform for linking the entry process of F. tularensis at the cell membrane to the cytoskeleton and the intracellular signaling pathways (Tamilselvam and Daehler, 2008).

To date, at least 268 gene products have been identified, that are important for replication of F. tularensis within mammalian cells (Table 1; Anthony et al., 1994; Baron and Nano, 1998; has not been identified. Elongation factor E2 is expressed on the surface of F. tularensis and bind to surface nucleolin expressed on the surface of macrophages (Barel et al., 2008). It is however not known which of these receptors are used predominantly in vivo.

When opsonized F. tularensis binds macrophages, it is engulfed in a unique asymmetric spacious pseudopod loops (Clemens et al., 2005). This unique mechanism of uptake has been shown to be dependent on intact CR3 and complement factor 3 (Clemens et al., 2005). Syk is important for Fcγ-mediated phagocytosis in macrophages and neutrophils (Greenberg et al., 1994; Raeder et al., 1999). Activation of Syk results in the activation of MAP kinase (ERKs) through Protein kinase C (PKC) leading to actin polymerization and induction of phagocytosis (Cox et al., 1996; Raeder et al., 1999). Syk has been shown to be important for the uptake of F. tularensis but the upstream receptor required for activation of Syk has not been identified (Parsa et al., 2008). Activation of Syk leads to subsequent activation of the Erk pathway but the direct binding partner of Syk is yet to be identified (Figure 2; Parsa et al., 2008). Interestingly, actin microfilament has been shown to be important for this process (Clemens et al., 2005).

![Figure 2](image-url)

**Figure 2** | Entry into and evasion of host cell innate immune response by *Francisella tularensis*. Phagocytosis of *F. tularensis* by macrophages is mediated by Syk-dependent activation of Erk, which likely triggers actin polymerization at the phagocytic cup. In addition, there is TLR2 dependent activation of Akt leading to induction of proinflammatory cytokines and phagosomal maturation. Akt activation is counteracted by SHIP activation, but the balance between the two opposingprocess is tilted toward escape of *F. tularensis* into the cytosol. Within the cytosol, *F. tularensis* activates both caspase-1 and caspase-3 but it is able to delay induction of apoptosis and pyroptosis through Ras and NFκB dependent anti-apoptotic mechanisms as well as AIM2-dependent inhibition of caspase-1 activation.
# Table 1 | List of intracellular growth defective mutants.

## PROTEINS OF UNKNOWN FUNCTION

| Protein ID | Description                                      |
|------------|--------------------------------------------------|
| FTN_0027   | Conserved protein of unknown function            |
| FTN_0041   | Protein of unknown function                      |
| FTN_0109   | Protein of unknown function                      |
| FTN_0132   | Protein of unknown function                      |
| FTN_0149   | Conserved protein of unknown function            |
| FTN_0275   | Conserved protein of unknown function            |
| FTN_0290   | Protein of unknown function                      |
| FTN_0297   | Conserved protein of unknown function            |
| FTN_0428   | Protein of unknown function                      |
| FTN_0444   | Membrane protein of unknown function             |
| FTN_0477   | Conserved protein of unknown function            |
| FTN_0788   | Conserved protein of unknown function            |
| FTN_0855   | Protein of unknown function                      |
| FTN_0915   | Conserved protein of unknown function            |
| FTN_0925   | Protein of unknown function                      |
| FTN_0930   | Protein of unknown function                      |
| FTN_0933   | Protein of unknown function                      |
| FTN_0977   | Conserved protein of unknown function            |
| FTN_1170   | Conserved protein of unknown function            |
| FTN_1172   | Conserved protein of unknown function            |
| FTN_1175   | Membrane protein of unknown function             |
| FTN_1256   | Membrane protein of unknown function             |
| FTN_1343   | Conserved protein of unknown function            |
| FTN_1367   | Protein of unknown function                      |
| FTN_1457   | Protein of unknown function                      |
| FTN_1542   | Conserved protein of unknown function            |
| FTN_1624   | Conserved protein of unknown function            |
| FTN_1696   | Protein of unknown function                      |
| FTN_1713   | Protein of unknown function                      |
| FTN_1764   | Protein of unknown function                      |

## HYPOTHETICAL PROTEINS

| Protein ID | Description                                      |
|------------|--------------------------------------------------|
| FTL_0439   | Hypothetical outer membrane protein              |
| FTL_0544   | Hypothetical protein; polyphosphate kinase       |
| FTL_0706   | Hypothetical membrane protein; LPS biosynthesis  |
| FTL_0886   | Conserved hypothetical protein YieA; possible tRNA-\(i\)G37 methylthiotransferase |
| FTL_1096   | Hypothetical lipoprotein; ABC transporter and potential disulfide bond formation |
| FTL_1414   | Hypothetical protein; possible capsule-related protein |
| FTL_0030   | Hypothetical membrane protein                    |
| FTL_0038   | Hypothetical protein                             |
| FTL_0169   | Conserved hypothetical membrane protein          |
| FTL_0336   | Hypothetical protein                             |
| FTL_0384   | Conserved hypothetical protein                    |
| FTL_0403   | Hypothetical membrane protein                    |
| FTL_0534   | Conserved hypothetical membrane protein          |
| FTL_0556   | Hypothetical protein                             |
| FTL_0696   | Hypothetical protein                             |
| FTL_0709   | Hypothetical protein                             |
| FTL_0727   | Hypothetical membrane protein                    |
| FTL_0759   | Conserved hypothetical protein                    |
| FTL_0792   | Hypothetical protein                             |
| FTL_0847   | Conserved hypothetical protein                    |

## METABOLIC PROTEINS

| Protein ID | Description                                      |
|------------|--------------------------------------------------|
| FTL_0028   | pryB Aspartate carbamoyltransferase              |
| FTL_0029   | carB Carbamoyl-phosphate synthase large chain    |
| FTL_0030   | carA Carbamoyl-phosphate synthase small chain    |
| FTL_0483   | glgB Glycogen branching enzyme, GlgB; polysaccharide metabolism |
| FTL_0592   | wbtA dTDP-glucose 4,6-dehydratase, WbtA, O-antigen polysaccharide biosynthesis |
| FTL_0594   | wbtC UDP-glucose-4-epimerase, WbtC, O-antigen polysaccharide biosynthesis |
| FTL_0606   | wbtM dTDP-glucose 4,6-dehydratase, WbtM, O-antigen polysaccharide biosynthesis |
| FTL_0766   | ggt Gamma-glutamyl transpeptidase; amino acid, arachidonic acid, and glutathione |
| FTL_0789   | aspC2 Aspartate aminotransferase; amino acid biosynthesis |
| FTL_1262   | chor A Chorismate family binding protein; aromatic amino acid, and folate biosynthesis |
| FTL_1415   | capC Capsule biosynthesis protein CapC           |
| FTL_1416   | capB Capsule biosynthesis protein CapB           |
| FTL_0020   | carB Carbamoyl-phosphate synthase large chain    |
| FTL_0035   | pyrF Orotidine-5-phosphate decarboxylase          |

(Continued)
| Gene  | Description                                                                 | Gene  | Description                                      |
|-------|------------------------------------------------------------------------------|-------|--------------------------------------------------|
| FTN_0036 | pyrD | Dihydroorotate oxidase                                                      | FTN_1121 | phrB | Deoxyribodipyrimidine photolyase                  |
| FTN_0063 | iVE | Branched-chain amino acid aminotransferase (class IV)                       | FTN_1131 | putA | Bifunctional proline dehydrogenase, pyrrole-5-carboxylate dehydrogenase |
| FTN_0090 | acpA | Acid phosphatase                                                            | FTN_1135 | arO | 3-Dehydroquinase synthetase                      |
| FTN_0111 | nbH | Riboflavin synthase beta-chain                                              | FTN_1222 | kpsF | Phosphoglucoisomerase                            |
| FTN_0113 | ribC | Riboflavin synthase alpha chain                                             | FTN_1231 | gloA | Lactoylglutathione lyase                         |
| FTN_0125 | adkA | Propionate kinase 2/acetate kinase A                                        | FTN_1233 | queA | Halaacid dehalogenase-like hydrolase             |
| FTN_0178 | purA | Adenylosuccinate synthetase                                                 | FTN_1234 | queA | S-adenosylmethionine: tRNA                        |
| FTN_0199 | oyoE | Heme O synthase                                                             | FTN_1234 | queA | Ribosyltransferase-isomerase                     |
| FTN_0211 | pcp | Pyrrolidine carboxylate peptidase                                            | FTN_1333 | ttkA | Transketolase I                                 |
| FTN_0218 | nfnB | Dihydropyridine reductase                                                   | FTN_1415 |      | Thioredoxin                                     |
| FTN_0319 |      | Amino acid–polyamine–organocation family protein                            | FTN_1417 | manB | Phosphomannomutase                              |
| FTN_0343 |      | Aminotransferase                                                            | FTN_1421 | wbtH | Glutamine amidotransferase/asparagine synthase   |
| FTN_0358 |      | tRNA-methylthiotransferase MiaB protein                                      | FTN_1428 | wbtO | Transferase                                      |
| FTN_0420 |      | SAICAR synthetase/ phosphoribosylamine-glycine ligase                       | FTN_1494 | aceE | Pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase |
| FTN_0483 |      | Bifunctional NMM adenylyltransferase/nudix hydroxylase                      | FTN_1523 |      | Amino acid–polyamine–organocation family protein |
| FTN_0486 | Slt | Soluble lytic murein transglycosylase                                        | FTN_1553 | nudH | dGTP pyrophosphohydrolase                        |
| FTN_0504 |      | Lysine decarboxylase                                                        | FTN_1557 |      | Oxidoreductase iron/ascorbate family protein     |
| FTN_0511 | gcvP1 | Glycine cleavage system P protein, subunit 1                               | FTN_1584 | glpD | GlyceroL-3-phosphate dehydrogenase               |
| FTN_0524 | Asd | Aspartate semialdehyde dehydrogenase                                        | FTN_1585 | glpK | Glycerol kinase                                 |
| FTN_0527 | thrC | Threonine synthase                                                          | FTN_1597 | prfC | Peptide chain release factor 3                   |
| FTN_0545 |      | Glycosyl transferase, group 2                                               | FTN_1619 | appC | Cytochrome bd-II terminal oxidase subunit I      |
| FTN_0567 |      | tRNA synthetase class II (D, K, and N)                                      | FTN_1620 | appB | Cytochrome bd-II terminal oxidase subunit II     |
| FTN_0588 |      | Asparaginase                                                                | FTN_1621 |      | Predicted NAD/FAD-dependent oxidoreductase       |
| FTN_0593 | sucD | Succinyl-CoA synthetase, alpha subunit                                       | FTN_1655 | rluC | Ribosomal large subunit pseudouridine synthase C |
| FTN_0598 |      | tRNA-dihydouridine synthase                                                 | FTN_1701 |      | Glutamate decarboxylase                          |
| FTN_0633 | katG | Peroxidase/catalase                                                         | FTN_1707 | rbsK | Ribokinase, pfkB family                         |
| FTN_0692 |      | Aminotransferase                                                            | FTN_1777 | trpG | Anthranilate synthase component II               |
| FTN_0695 | Add | Deoxyadenosine deaminase/adenosine deaminase                                 | FTT0203c | purH | Bifunctional purine biosynthesis protein         |
| FTN_0746 |      | tRNA-adenylyltransferase subunit 2                                         | FTT0204 | purA | Adenylosuccinate synthetase                      |
| FTN_0806 |      | Sulfate adenylyltransferase subunit 2                                       | FTT0435 | Ctu | Citrulline ureidase                              |
| FTN_0811 |      | 3-Phosphoshikimate 1-carboxyvinyl transferase                               | FTT0588 | aroA | 3-Phosphoshikimate 1-carboxyvinyl transferase   |
| FTN_0822 |      | Succinyl-CoA synthetase, alpha subunit                                       | FTT1234 |      | Choloylglycine hydroxylase family protein        |
| FTN_0840 | mdaB | NADPH-quinone reductase (modulator of drug activity B)                      | FTT1665 | purL | Aspartate carboxamoyltransferase                 |
| FTN_0877 | cls | Cardiolipin synthetase                                                      | FTT1712c | purF | Phosphoribosylformylglycinamidin synthase        |
| FTN_0928 |      | ATP-dependent protease HslVU, peptidase subunit                              | FTT1721c | purF | (Amidophosphoribosyltransferase)2               |
| FTN_0954 |      | Histidine acid phosphatase                                                  | FTT1762c |      | Acetyltransferase protein                       |
| FTN_0957 |      | Short chain dehydrogenase                                                   | FTL_0101 | guaA | GMP synthase (glutamine-hydrolyzing)            |
| FTN_0965 |      | Metal-dependent exopeptidase                                                | FTL_0107 | guaA | Inosine-5-monophosphate dehydrogenase           |
| FTN_0963 |      | Bifunctional protein: glutaredoxin 3/ribonucleotide reductase beta subunit  |
| FTN_0983 |      | 3-Phosphoshikimate 1-carboxyvinyl transferase                               |
| FTN_0983 | prmA | 50S ribosomal protein L1, methyltransferase                                 |
| FTN_0995 | hslV | ATP-dependent protease HslVU, peptidase subunit                              |
| FTN_1018 |      | Aldolase/adducin class II family protein                                     |
| FTN_1046 | wzb | Low molecular weight (LMW) phosphotyrosine protein phosphatase               |
| FTN_1061 |      | Acid phosphatase, HAD superfamily protein                                   |

**TRANSPORTER PROTEINS**

| Gene  | Description                                                                 |
|-------|------------------------------------------------------------------------------|
| FTL_0304 | Na+/H+ antiporter; regulation of pH                                         |
| FTL_0837 | o-Methionine transport protein (ABC transporter), MetIQ                      |
| FTL_0838 | o-Methionine transport protein (ABC transporter), MetN                      |
| FTL_1583 | XasA Glutamate–aminobutyric acid antiporter, XasA; amino acid transport    |
| FTL_1806 | Major facilitator superfAMILY transporter                                    |
| FTL_0008 | 10TMS drug/metabolite exporter protein                                        |
| FTL_0018 | sdaC Serine permease                                                         |

(Continued)
### Table 1 | Continued

| Gene | Description |
|------|-------------|
| FTN_0566c | Major facilitator superfamily (MFS) transport protein |
| FTT0129 | Major facilitator superfamily (MFS) transport protein |
| DNA MODIFYING |  |
| FTL_0878 | DNA/RNA endonuclease family |
| FTL_0133 | Ribonuclease II family protein |
| FTL_0287 | Type I restriction-modification system, subunit R (restriction) |
| FTL_0577 | mutL | DNA mismatch repair enzyme with ATPase activity |
| FTL_0680 | uvrC | Excinuclease ABC, subunit C |
| FTL_0710 | Type I restriction-modification system, subunit R (restriction) |
| FTL_0838 | xthA | Exodeoxyribonuclease III |
| FTL_1017 | Pseudogene: DNA-3-methyladenine glycosylase |
| FTL_1027 | ruvC | Holliday junction endodeoxyribonuclease |
| FTL_1073 | DNA/RNA endonuclease G |
| FTL_1154 | Type I restriction-modification system, subunit S |
| FTL_1176 | uvrB | Excinuclease ABC, subunit B |
| FTL_1197 | recR | RecFOR complex, RecR component |
| FTL_1293 | mhB | Ribonuclease HII |
| **TRANSCRIPTION/TRANSLATION** |  |
| FTL_1357 | recB | ATP-dependent exoDNase_subunit |
| FTN_1487 | Restriction endonuclease |
| **CELL DIVISION** |  |
| FTL_1542 | migR | Macrophage intracellular growth regulator |
| FTL_1606 | sspA | Stringent starvation protein A/regulator of transcription |
| FTL_1914 | ripA | Required for intracellular proliferation, factor A |
| FTL_0480 | fevR | Francisella effector of virulence regulation |
| FTL_0567 | rRNA synthetase class II (D, K, and N) |
| FTL_0598 | tRNA-dihydrouridine synthase |
| FTL_1290 | mgIA | Macrophage growth locus, protein A |
| FTL_1291 | mgIB | Macrophage growth locus, subunit B |
| FTL_1412 | DNA-directed RNA polymerase subunit |
| FTL_0552 | Transcriptional response regulator |
| **TYPE IV PILIN** |  |
| FTL_0415 | pilA | Type IV pilus, pilus assembly protein |
| FTL_1137 | pilO | Type IV pilus secretin component |
| FTL_1139 | pilO | Type IV pilus glycosylation protein |
| **OTHERS** |  |
| FTL_0094 | clpB | ClpB protein |
| FTL_1670 | dsbB | Disulfide bond formation protein, DsbB |
| FTL_0107 | lepA | GTP-binding protein LepA |
| FTL_0155 | Competence protein |
| FTL_0182 | ATP-binding cassette (ABC) superfamily protein |
| FTL_0286 | Transposase |
| FTL_0338 | MutT/nudix family protein |
| FTL_0465 | Sua5/YycO/YrdC family protein |
| FTL_0646 | cscK | ROK family protein |
| FTL_0672 | secA | Preprotein translocase, subunit A (ATPase, RNA helicase) |
| FTL_0708 | tspO | Tryptophan-rich sensory protein |
| FTL_0985 | D1/Pfpl family protein |
| FTL_1002 | blaA | Beta-lactamase class A |
| FTL_1031 | ftnA | Ferric iron binding protein, ferritin-like |
| FTL_1034 | rnfB | Iron-sulfur cluster-binding protein |
| FTL_1058 | tig | Trigger factor (TF) protein |
| FTL_1064 | PhoH family protein, putative ATPase |
| FTL_1145 | era | GTP-binding protein |
| FTL_1217 | ATP-binding cassette (ABC) superfamily protein |
| FTL_1240 | BoA family protein |
| FTL_1241 | DedA family protein |
| FTL_1263 | comL | Competence lipoprotein ComL |
| FTL_1355 | Regulatory factor, Bvg accessory factor family |
| FTL_1518 | relA | GDP pyrophosphokinase/GTP pyrophosphokinase |
| FTT0029c | figA | Francisella iron regulated gene A |
| FTT0918 |  |
| FTL_0380 | sodC | Superoxide dismutase (Cu–Zn) precursor |
| FTL_0439 | fupA/B | Siderophore biosynthesis |
MODULATION OF PHAGOSOME BIOGENESIS

Phagosomal maturation involves sequential interaction between the nascent phagosome and the endocytic and lysosomal vesicles resulting in the conversion of the phagosome to a phagolysosome within which the bacterium or a particle is degraded (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002). After biogenesis from the plasma membrane, the nascent phagosome fuses with vesicles from the early endosome in a process that is regulated by Rab5 GTPase and the downstream effector early endosomal antigen 1 (EEA1). This is followed by interaction with the late endosome that is controlled by Rab7 GTPase. The late endosome-like phagosome becomes acidified through acquisition of the vacuolar ATPase, which pumps protons into the lumen of the phagosome resulting in acidification of the lumen. The acidified phagosome subsequently fuses to the lysosomes to form a phagolysosome, which is very rich in acid hydrolases. Within this compartment the microbe or particle is degraded (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002; Figure 1). The maturation process is very rapid and is completed within 15–30 min of phagosome biogenesis from the plasma membrane (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002).

Different intracellular pathogens have evolved different mechanisms to subvert the default endocytic maturation to create permissive niches that allow intracellular replication (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002). The strategies include (i) Arrest of phagosome maturation at a distinct stage in the endosomal–lysosomal degradation pathway, as occurs in infection with Legionella pneumophila; (ii) Survival and replication within an acidic environment of a mature phagolysosome, as exemplified by Coxiella burnetii; and (iii) Replication within the cytosol after degradation of the phagosomal membrane, as occurs in infection with Listeria monocytogenes and Shigella flexneri (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002).

Although the endocytic maturation stage of phagosome harboring vacuolar pathogens has been classified into early or late endosome, maturation of phagosome harboring intracellular pathogens is aberrant and is not a classical full maturation of any of the defined endocytic stages (Santic et al., 2010a). For example, the Mycobacterium tuberculosis phagosome acquires Rab5 but lacks several downstream effectors of Rab5 that are present on mature early endosome. It also acquires procathepsin D, which is the immature form of the lysosomal enzyme cathepsin D (Sturgill-Koszycki et al., 1996; Derre and Isberg, 2004). Therefore, it might be more accurate to classify phagosome of intracellular vacuolar pathogens as early endosome-like or late endosome-like phagosome (Santic et al., 2010a).

The FCP transiently acquires the EEA1 followed by the acquisition of the late endosomal markers, Lamp1/2, Cd63, and Rab7 as well as the vacuolar ATPase, which acidifies the phagosome (Figure 1; Golovliov et al., 2003b; Clemens et al., 2004; Santic et al., 2005a,b, 2007, 2008; Checroun et al., 2006; Bonquist et al., 2008; Qin et al., 2009). The FCP does not however co-localize with the lysosomal acid hydrolase cathepsin D and the fluid face marker, lysotracker

Gray et al., 2002; Golovliov et al., 2003a; Nano et al., 2004; Santic et al., 2005b, 2007; Twine et al., 2005; Deng et al., 2006; Pechous et al., 2006, 2008; Tempel et al., 2006; Charity et al., 2007; de Bruin et al., 2007; Maier et al., 2007; Mohapatra et al., 2007a,b, 2008; Raynaud et al., 2007; Bonquist et al., 2008; Brotcke and Monack, 2008; Fuller et al., 2008; Meibom et al., 2008; Sammons-Jackson et al., 2008; Alkhuder et al., 2009; Dean et al., 2009; Mahawar et al., 2009; Santiago et al., 2009; Schulert et al., 2009; Ahlund et al., 2010; Asare and Abu Kwaik, 2010; Jia et al., 2010; Sen et al., 2010). These include the Francisella pathogenicity Island (FPI) proteins IgIA, IgIB, IgIC, IgID, pdpA, pdpB, pdpD and their regulators, MglA, SspA, FevR, MigR, RipA, PigR, and PmrA (Baron and Nano, 1998; Gray et al., 2002; Golovliov et al., 2003a; Charity et al., 2007, 2009; de Bruin et al., 2007; Mohapatra et al., 2007b; Bonquist et al., 2008; Brotcke and Monack, 2008; Fuller et al., 2008; Buchan et al., 2009). The FPI is composed of 17 genes and recent mutagenesis experiments have shown that most of the genes are important for survival within the host cell (Golovliov et al., 2003a; de Bruin et al., 2007; Barker et al., 2009; Broms et al., 2009; Schmerk et al., 2009). Some of the gene products on the FPI form a type VI-like secretion system through which effector proteins are injected into the host cell cytosol to modulate biogenesis of the FCP and to enable the bacterium to disrupt the phagosome membrane and escape into the cytosol (Golovliov et al., 2003a; Santic et al., 2007; Barker et al., 2009; Broms et al., 2009; Schmerk et al., 2009). MglA, SspA, and PmrA bind cooperatively with RNA polymerase to regulate a large number of genes including those of the FPI (Brotcke et al., 2006; Charity et al., 2007; Mohapatra et al., 2007b; Bell et al., 2010) that are important for survival within the host cell. This regulation is mediated in part by FevR which is important for escape and replication within the cytosol (Brotcke and Monack, 2008). FevR is also independently regulated by MigR (Buchan et al., 2009) indicating that FevR plays a central role in the regulation of virulence in F. tularensis. Independent of FevR, MglA, and SspA also regulate virulence genes through cooperative interaction with PigR and the alamone ppGpp (Charity et al., 2009). Whereas most of the genes regulated by the different pathways are common, there are subsets of genes that are regulated independently by the different pathways (Brotcke et al., 2006; Charity et al., 2007; Mohapatra et al., 2007b). A large number of these gene products and most of the proteins that are necessary for escape and replication are hypothetical proteins. It is conceivable to speculate that some of these gene products constitute effector proteins that are secreted by the type VI secretion-like system. Also important for intracellular replication are genes involved in the transport of metabolic intermediates and different metabolic pathways including amino acid metabolism, nucleotide metabolism, and carbohydrate metabolism (Pechous et al., 2006; Alkhuder et al., 2009; Mahawar et al., 2009; Schulert et al., 2009; Asare and Abu Kwaik, 2010). The large number of metabolic genes that is required for replication indicates that the FCP is replete of nutrients and F. tularensis require de novo synthesis in order to survive and replicate within the host cells. Once in the cytosol where nutrient is readily available, the FCP is replete of nutrients and escape into the cytosol to modulate biogenesis of the FCP and to enable the bacterium to disrupt the phagosome membrane and escape into the cytosol (Golovliov et al., 2003a; Santic et al., 2007; Barker et al., 2009; Broms et al., 2009; Schmerk et al., 2009). MglA, SspA, and PmrA bind cooperatively with RNA polymerase to regulate a large number of genes including those of the FPI (Brotcke et al., 2006; Charity et al., 2007; Mohapatra et al., 2007b; Bell et al., 2010) that are important for survival within the host cell. This regulation is mediated in part by FevR which is important for escape and replication within the cytosol (Brotcke and Monack, 2008). FevR is also independently regulated by MigR (Buchan et al., 2009) indicating that FevR plays a central role in the regulation of virulence in F. tularensis. Independent of FevR, MglA, and SspA also regulate virulence genes through cooperative interaction with PigR and the alamone ppGpp (Charity et al., 2009). Whereas most of the genes regulated by the different pathways are common, there are subsets of genes that are regulated independently by the different pathways (Brotcke et al., 2006; Charity et al., 2007; Mohapatra et al., 2007b). A large number of these gene products and most of the proteins that are necessary for escape and replication are hypothetical proteins. It is conceivable to speculate that some of these gene products constitute effector proteins that are secreted by the type VI secretion-like system. Also important for intracellular replication are genes involved in the transport of metabolic intermediates and different metabolic pathways including amino acid metabolism, nucleotide metabolism, and carbohydrate metabolism (Pechous et al., 2006; Alkhuder et al., 2009; Mahawar et al., 2009; Schulert et al., 2009; Asare and Abu Kwaik, 2010). The large number of metabolic genes that is required for replication indicates that the FCP is replete of nutrients and F. tularensis require de novo synthesis in order to survive and replicate within the host cells. Once in the cytosol where nutrient is readily available, the FCP may acquire nutrients through the importation of metabolic intermediates from the host cell cytosol. This may explain why mutations in a large number of metabolic intermediate transporters block bacterial escape into the cytosol (Table 1; Qin and Mann, 2006; Maier et al., 2007; Asare and Abu Kwaik, 2010).
Within 30–60 min, the bacterium disrupts the phagosomal membrane and escapes into the host cell cytosol (Figure 1; Chong et al., 2008; Santic et al., 2008). Acidification of the vacuole is important for the ability of the bacteria to escape into the cytosol, since inhibition of the vATPase results in a delay in bacterial escape into the cytosol (Chong et al., 2008; Santic et al., 2008). *F. tularensis* has been shown to escape into the cytosol in different cell types including macrophages and neutrophils (Figure 1; Golovliov et al., 2003a; Clemens et al., 2004; Santic et al., 2005a; McCaffrey and Allen, 2006). It has not been determined if *F. tularensis* arrest phagosome maturation before escaping into cytosol or if the bacterium manages to escape before the phagosome fuses to the lysosome. However, trafficking of the migR and fevR mutants of *F. tularensis* in macrophages suggests that there is arrest of phagosome biogenesis prior to bacterial escape into the cytosol (Buchan et al., 2009). Comparison of trafficking of the migR and fevR mutants showed that whereas the fevR mutant is trapped in a LAMP1–positive compartment, the phagosome containing the migR mutant matures into a phagolysosome enriched in LAMP1 and cathepsin D (Buchan et al., 2009). Conversely, data from studies of *F. tularensis* trafficking in neutrophils suggest that a fraction of the phagosome of wild-type bacteria that are unable to escape end up in a phagolysosome. This may suggest that *F. tularensis* does not inhibit phagosome maturation in neutrophils but rather escape into the cytosol before the phagosome matures into a phagolysosome (McCaffrey and Allen, 2006). Interestingly, arrest in phagosome biogenesis and rapid escape of *F. tularensis* into the cytosol is also exhibited in arthropod vector–derived cells, indicating exploitation of conserved eukaryotic processes by *F. tularensis* to infect and proliferate within arthropod and mammalian cells (Santic et al., 2009).

**ESCAPE INTO THE CYTOSOL**

Like other intracellular pathogens, *F. tularensis* must overcome the host innate immune response to successfully colonize the intracellular niche. Since the primary host defense is centered on the antimicrobial properties of the phagosome, *F. tularensis* like other cytosolic bacteria escapes from the phagosome into the cytosol where it replicates (Goebel and Kuhn, 2000; Golovliov et al., 2003a; Clemens et al., 2004; Santic et al., 2005a; McCaffrey and Allen, 2006; Ray et al., 2009). In order to escape into the cytosol, the FCP transiently acquires the vacuolar ATPase, which acidifies the phagosome followed by rapid escape of *F. tularensis* to the cytosol (Figure 1; Golovliov et al., 2003b; Clemens et al., 2004; Santic et al., 2005a,b, 2007, 2008; Checroun et al., 2006; Bonquist et al., 2008; Qin et al., 2009). The acidification is important since inhibition of the vATPase by bafilomycin A delays escape of the bacterium into the cytosol indicating that there is a factor involved in disruption of the phagosome that is expressed or activated at acidic pH. Between 15 and 30 min of residence in the phagosome in human macrophages, the bacteria begin to escape into the cytosol (Figure 1; Santic et al., 2010a). It is within the cytosol that the bacteria replicate (Figure 1). The mechanism by which the bacterium escapes into the cytosol is not well understood.

Unlike *L. monocytogenes*, a large number of genes have been shown to be important for escape of *F. tularensis* into the host cell cytosol (Table 2; Golovliov et al., 2003a; Santic et al., 2005b; Table 2 | List of escape defective mutants.

| Table 2 | List of escape defective mutants. |
| PROTEINS OF UNKNOWN FUNCTION | |
| FTN_0027 | Conserved protein of unknown function |
| FTN_0109 | Protein of unknown function |
| FTN_0149 | Conserved protein of unknown function |
| FTN_0297 | Conserved protein of unknown function |
| FTN_0444 | Membrane protein of unknown function |
| FTN_0788 | Conserved protein of unknown function |
| FTN_0865 | Protein of unknown function |
| FTN_0915 | Conserved protein of unknown function |
| FTN_0925 | Protein of unknown function |
| FTN_0930 | Protein of unknown function |
| FTN_0933 | Protein of unknown function |
| FTN_0977 | Conserved protein of unknown function |
| FTN_1175 | Membrane protein of unknown function |
| FTN_1256 | Membrane protein of unknown function |
| FTN_1343 | Conserved protein of unknown function |
| FTN_1624 | Conserved protein of unknown function |
| FTN_1764 | Protein of unknown function |
| HYPOTHETICAL PROTEINS | |
| FTN_0030 | Hypothetical membrane protein |
| FTN_0038 | Hypothetical protein |
| FTN_0096 | Conserved hypothetical membrane protein |
| FTN_0403 | Hypothetical membrane protein |
| FTN_0727 | Hypothetical membrane protein |
| FTN_0792 | Hypothetical protein |
| FTN_0847 | Conserved hypothetical protein |
| FTN_1098 | Conserved hypothetical membrane protein |
| FTN_1349 | Hypothetical protein |
| FTN_1395 | Conserved hypothetical protein |
| FTN_1406 | Conserved hypothetical membrane protein |
| FTN_1612 | Hypothetical protein |
| FTN_1686 | Hypothetical membrane protein |
| FTT1103 | Conserved hypothetical lipoprotein |
| FPI PROTEINS | |
| FTN_1309 | pdpA | Protein of unknown function |
| FTN_1313 | vgrG | |
| FTN_1317 | igII | Intracellular growth locus, subunit I |
| FTN_1322 | igIC | Intracellular growth locus, subunit C |
| FTN_1323 | igIB | Intracellular growth locus protein B |
| FTN_1324 | igIA | Intracellular growth locus A |
| FTN_1325 | pdpD | Protein of unknown function |
| METABOLIC PROTEINS | |
| FTN_0019 | pyrB | Aspartate carbamoyltransferase |
| FTN_0063 | ivE | Branched-chain amino acid aminotransferase protein (class IV) |
| FTN_0090 | | Acid phosphatase |
| FTN_0125 | ackA | Propionase kinase 2/acetate kinase A |
| FTN_0483 | Bifunctional NMN adenylyltransferase/Nudix hydrolase |
| FTN_0504 | Lysine decarboxylase |
| FTN_0511 | Shikimate 5-dehydrogenase |
| FTN_0524 | asd | Aspartate semialdehyde dehydrogenase |

(Continued)
Table 2  |  Continued

| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| FTN_0527 | thrC  Threonine synthase                                                    |
| FTN_0545 | Glycosyl transferase, group 2                                               |
| FTN_0692 | nadA Quinolinate synthetase A                                               |
| FTN_0746 | air  Alanine racemase                                                        |
| FTN_0811 | birA Biotin–acyetyl-CoA-carboxylase ligase                                   |
| FTN_0822 | p-Aminobenzoate synthase component I                                        |
| FTN_0840 | mdaB NADPH-quinone reductase (modulator of drug activity B)                 |
| FTN_0877 | cls  Cardiolipin synthetase                                                  |
| FTN_0954 | Histidine acid phosphatase                                                   |
| FTN_0965 | Metal-dependent exopeptidase                                                 |
| FTN_0983 | Bifunctional protein: glutaredoxin 3/ribonucleotide reductase beta subunit  |
| FTN_0988 | prmA 50S ribosomal protein L11, methyltransferase                            |
| FTN_1061 | Acid phosphatase, HAD superfamily protein                                   |
| FTN_1222 | kpsF  Phosphosugar isomerase                                                 |
| FTN_1231 | gaoA  Lactoylglutathione lyase                                               |
| FTN_1234 | queA  S-adenosylmethionine: tRNA ribosyltransferase-isomerase                |
| FTN_1333 | tktA  Transketolase I                                                       |
| FTN_1376 | manC  Serine permease                                                        |
| FTN_1418 | AcpB, AcpC, and HAP) of which are also found in the virulent                |
| FTN_1428 | wbTO  Transf erase                                                          |
| FTN_1494 | aceE  Pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase  |
| FTN_1553 | nudH  dGTP pyrophosphohydrolase                                              |
| FTN_1597 | prfC  Peptide chain release factor 3                                        |
| FTN_1621 | Predicted NAD/FAD-dependent oxidoreductase                                  |
| FTN_1655 | ruC  Ribosomal large subunit pseudouridine synthase C                        |
| FTN_0624 | Serine permease                                                             |
| FTN_0728 | Predicted Co/Zn/Cd cation transporter                                        |
| FTN_0997 | Proton-dependent oligopeptide transporter (POT) family protein, dv, or tripeptide: H+ symporter |
| FTN_1344 | Major facilitator superfamily (MFS) transport protein                       |
| FTN_1611 | Major facilitator superfamily (MFS) transport protein                       |
| FTN_1711 | tyrP  Tyrosine permease                                                     |

DNA MODIFICATION

| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| FTN_0133 | Ribonuclease II family protein                                               |
| FTN_0680 | uvrC  Excinuclease ABC, subunit C                                            |
| FTN_0710 | Type I restriction–modification system, subunit R (restriction)             |
| FTN_1027 | ruvC  Holliday junction endodeoxyribonuclease                                |
| FTN_1073 | DNA/RNA endonuclease G                                                       |
| FTN_1154 | Type I restriction–modification system, subunit S                            |

TRANSCRIPTION/TRANSLATION

| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| FTN_1290 | mglA  Macrophase growth locus, protein A                                      |
| FTL_1542 | migR  Macrophase intracellular growth regulator                              |
| FTL_1914 | ripA  Required for intracellular proliferation, factor A                    |
| FTN_0480 | fevR  Francisella effector of virulence regulation                           |

TYPE IV PILIN

| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| FTN_1137 | pilQ  Type IV pili secretion component                                       |
| FTN_1139 | pilQ  Type IV pili glycosylation protein                                     |

OTHERS

| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| FTN_0286 | Transposase                                                                 |
| FTN_0646 | cscK  ROK family protein                                                     |
| FTN_0768 | tspO  Tryptophan-rich sensory protein                                         |
| FTN_1034 | rnfB  Iron–sulfur cluster-binding protein                                     |
| FTN_1145 | era  GTP-binding protein                                                     |
| FTN_1241 | DedA family protein                                                         |
| FTN_1263 | comL  Competence lipoprotein ComL                                            |
| FTN_1453 | Two-component regulator, sensor histidine kinase                             |
| FTN_1518 | relA  GDP pyrophosphokinase/GTP pyrophosphokinase                           |

Qin and Mann, 2006; Mohapatra et al., 2008; Barker et al., 2009; Broms et al., 2009; Buchan et al., 2009; Schmerk et al., 2009; Schulert et al., 2009; Asare and Abu Kwaik, 2010). Recent mutagenesis experiments have shown that most of the genes of the FPI that form the type VI-like secretion system, affect escape of the bacterium into the cytosol and subsequent replication (Golovliov et al., 2003a; de Bruin et al., 2007; Barker et al., 2009; Broms et al., 2009; Schmerk et al., 2009). In contrast, IglD has been shown to be important for replication of the bacteria within the cytosol without any effect on phagosomal escape of the bacterium (Santic et al., 2007). The FPI protein VgrG has been shown to be a component of the secretory system as well as a substrate of the system (Barker et al., 2009). Unlike VgrG, IglI is a substrate of the type VI secretion system with no effect on the secretion apparatus. Both genes are important for escape of F. tularensis into the host cell cytosol but the mechanism of action has not been elucidated (Barker et al., 2009). The FPI proteins IglA, IglC, and pdpA are also required for escape of F. tularensis into the host cells cytosol but it has not been determined if they are secreted substrates or component of the type VI-like secretion apparatus.

Mutations in MglA and FevR negatively affect the ability of F. tularensis to escape from the phagosome into the cytosol (Santic et al., 2005b; Bonquist et al., 2008; Buchan et al., 2009). In contrast, MigR mutant behaves similar to the IglD mutant, which escapes but is unable to replicate within the cytosol indicating that MigR regulate genes that are important for replication within the cytosol (Santic et al., 2007; Buchan et al., 2009). Other genes that play critical roles in the escape of bacteria into the host cell cytosol include genes involved in DNA modification, transcription and translation, type II secretion, metabolic genes as well as genes involved in the transport of nutrients (Schulert et al., 2009; Asare and Abu Kwaik, 2010).

Although hemolytic activity has been observed in F. tularensis subspecies novicida and F. philomiragia (Lai et al., 2003), no hemolysin homolog has been identified in all the sequenced Francisella genome to date including those of novicida and philomiragia. There are between four and eight acid phosphatases in the Francisella genome depending on the subspecies. There are eight acid Phosphatases in the subspecies novicida genome, four (AcpA, AcpB, AcpC, and HAP) of which are also found in the virulent subspecies tularensis genome (Mohapatra et al., 2008). AcpA has
been shown to possess lipase activity (Mohapatra et al., 2007a), but all three Acp molecules are predicted to possess phosphoric ester hydrolase activity. Independent studies have shown that mutations in AcpA, AcpC, and HAP result in delay or inhibition of escape into the cytosol and reduced replication within human macrophages (Mohapatra et al., 2007a; Asare and Abu Kwaik, 2010), and that combined deletion of AcpA, AcpB, AcpC, and HAP results in complete inhibition of phagosomal escape and replication of subspecies novicida in the cytosol (Mohapatra et al., 2008). However, there is contradictory data on the role of these acid phosphatases in escape and intracellular replication. For example, Baron et al. (1999) have shown that AcpA in subspecies novicida is not important for replication within mouse macrophages. The difference between the role of AcpA in various subspecies may be due to the difference in the macrophages used, since trafficking of F. tularensis has been shown to be slightly different in mouse and human macrophages (Clemens et al., 2004; Checroun et al., 2006). Similarly, Child et al. (2010) have shown that combined deletion of AcpA-C does not affect the phagosomal escape or replication of the virulent subspecies tularensis within human macrophages. This indicates that there may be subtle differences in the mechanisms used by the different subspecies to escape into the host cell cytosol. There are numerous genes identified to be important for escape of F. tularensis that are designated as hypothetical proteins or proteins with unknown functions (Asare and Abu Kwaik, 2010). Some of these may be potential substrates for the type VI-like secretion system and identifying and characterizing them will help us to understand how F. tularensis modulates biogenesis of its phagosome and escape into the cytosol.

**MODULATION OF INFLAMMATORY RESPONSE TO INFECTION BY F. TULARENSIS**

The transcription factor NF-κB is involved in the regulation of inflammation by activating the induction of different proinflammatory cytokines (Lawrence, 2009). NF-κB represents a family of homo and heterodimer transcription factors, and the p65/p50 heterodimer is the most predominant active complex in mammalian cells (Burstein and Ducket, 2003). In resting cells, NF-κB proteins are predominantly sequestered in the cytoplasm by the NF-κB inhibitory proteins (IκBs; Karin and Ben-Neriah, 2000). The IκB kinase mediates phosphorylation of IκBs, followed by ubiquitination and proteasomal degradation, which is crucial to the activation and nuclear translocation of NF-κB (Karin and Ben-Neriah, 2000).

Early during infection when F. tularensis is localized within the phagosome, it activates the inflammatory response in macrophages by inducing the secretion of TNF-α in TLR-2 dependent manner (Figure 2; Telepnev et al., 2005; Katz et al., 2006). Induction of TNF-α secretion is mediated by the PI3K/Akt pathway, which also leads to activation of NF-κB (Telepnev et al., 2003; Katz et al., 2006; Parsa et al., 2006; Rajaram et al., 2006). Activation of NF-κB results in the induction and secretion of proinflammatory cytokines that restrict the escape of F. tularensis from the phagosome into the cytosol and promotes fusion of the FCP with the lysosome (Figure 2; Rajaram et al., 2009). Concomitant with escape into the cytosol, F. tularensis down-regulates NF-κB activation and TNF-α, IL-6, IL-8, and IL-12 secretion within 5 h post-infection, since the IgIC mutant which is unable to escape into the cytosol, does not down-regulate TNF-α, IL-6, IL-8, and IL-12 secretion (Figure 2; Telepnev et al., 2003, 2005).

The activation of PI3K/Akt pathway is negatively regulated by the Src homology 2 (SH2) domain-containing inositol-5’-phosphatase (SHIP) protein, since deficiency in SHIP expression results in enhanced Akt activation and NF-κB-driven transcription of proinflammatory cytokines, which promote fusion of the FCP with the Lysosome (Figure 2; Parsa et al., 2006; Rajaram et al., 2009). Conversely, over expression of SHIP leads to a decrease in NF-κB activation (Parsa et al., 2006). It is unknown how the delicate balance of Akt and SHIP activation is tilted toward SHIP promoted escape of F. tularensis into the cytosol. It will be interesting to determine how F. tularensis activates SHIP and the relations between SHIP activation and the disruption of the phagosome membrane that allow F. tularensis to escape into the cytosol (Figure 2). Once inside the cytosol F. tularensis induces Sp-1/Sp-3 dependent Fas expression that results in activation of caspase-3 and host cell death (Rajaram et al., 2009).

Cytosolic localization of F. tularensis in mouse macrophages results in type I interferon (IFN-I) and AIM2 dependent activation of the inflammasome (Figure 2; Mariathasan et al., 2005; Gavrilin et al., 2006; Henry et al., 2007; Fernandes-Alnemri et al., 2010; Jones et al., 2010). Cytosolic bacteria induce IRF-3 dependent activation of IFN-I, which in turn increases the expression of AIM2 (Figure 2; Jones et al., 2010). AIM2 recognize F. tularensis lipid/polysaccharide (MOP) transporter protein, MviN, which is homologous to the E. coli putative lipid II flippase, has recently been shown to suppress the induction of AIM2 (Ulland et al., 2010), since a mutation in the gene results in increase induction of AIM2 inflammasome-dependent IL-1β secretion and cytotoxicity in macrophages (Ulland et al., 2010). In addition to MviN, two other genes FTT_0584, with no characterized orthologs, and FTT_0748, which is homologous to the IclR family of transcriptional regulators, have been shown to suppress caspase-1 and ASC dependent secretion of IL-1β, since mutations in these genes resulted in hyper secretion of IL-1β (Weiss et al., 2007). Since AIM2 is not present in human macrophages, inflammasome mediators are likely to be different from the one described for mouse macrophages.

**ACTIVATION AND CONTROL OF HOST CELL APOPTOSIS**

Between 6 and 12 h post-infection, F. tularensis induce caspase-3 activation within the host cells, which culminate in the induction of apoptosis (Lai and Sjostedt, 2003; Santic et al., 2010b). F. tularensis LVS induces apoptosis in the J774A.1 murine macrophage cell
line through a pathway partly resembling the intrinsic apoptotic pathway (Lai and Sjostedt, 2003). The induction of apoptosis involves the release of mitochondrial cytochrome C into the cytosol with concomitant activation of caspase-9 and caspase-3 but not caspase-1, caspase-8, Bcl-2, or Bid (Lai and Sjostedt, 2003). In contrast, another study has shown that F. tularensis induces Sp-1/ Sp-3 activation of Fas in RAW 264.7 murine macrophage cells line, which results in activation of caspase-3, suggesting that F. tularensis induce apoptosis through the extrinsic pathway (Figures 1 and 2; Rajaram et al., 2009). Interestingly, infection of murine macrophages by F. tularensis has been shown to induce apoptotic cell death through down-regulation of activation of p38 MAPK compared to uninfected cells, but the mechanism of induction is yet to be defined (Hrstka et al., 2005). Although caspase-3 activation occurs early during infection in non-activated macrophages, it is not until about 18 h post-infection before there is induction of apoptosis, which is likely due to triggering anti-apoptotic processes (Figures 1 and 2; Lai and Sjostedt, 2003; Al-Khodor and Abu Kwaik, 2010; Santic et al., 2010b).

**HOST FACTORS REQUIRED INTRACELLULAR GROWTH OF F. TULARENSIS**

NF-κB plays a crucial role in regulation of apoptosis by triggering expression of various anti-apoptotic genes (Burstein and Duckett, 2003). We have shown that in order to maintain viability of the infected cell and allow F. tularensis to survive, there is simultaneous activation of caspases and NF-κB creating a delicate balance between them to maintain cell viability that is necessary for proliferation of the bacterium. Activation of NF-κB involves 1kB kinase-mediated phosphorylation of 1kB, followed by ubiquitination and proteasomal degradation (Karin and Ben-Neriah, 2000). Interestingly, in activated macrophages, F. tularensis elicits ubiquitin-dependent MHC class II down-regulation and degradation, thus compromising antigen presentation by macrophages to CD4 T cells (Wilson et al., 2009). It is not surprising that two ubiquitin proteins, the ubiquitin hydrolase USP22, and the ubiquitin ligase CDC27 has been shown to be important for replication of F. tularensis in human macrophages (Akimana et al., 2010).

It has been shown that F. tularensis triggers activation of Ras through the recruitment of PKCα and PKCβ-1 to the SOS2/Grb2 complex (Figure 2; Al-Khodor and Abu Kwaik, 2010). Silencing of SOS2, Grb2, PKCα, and PKCβ-1 is associated with rapid early activation of caspase-3 but does not affect phosphorylation of Akt or Erk (Al-Khodor and Abu Kwaik, 2010). This indicates that F. tularensis utilizes two independent mechanisms to modulate caspase-3 activity in order to survive inside host cells till the terminal stages of infection when induction of apoptosis leads to cell lysis and release of bacteria to the extracellular milieu. The bacterial factor necessary for the activation of NF-κB and Ras are yet to be identified.

**CONCLUDING REMARKS AND FUTURE DIRECTIONS**

Upon infection with F. tularensis, the host cells employ a myriad of arsenal to try to limit proliferation of the bacteria. The host cells activate signaling pathways to try to restrict escape of F. tularensis into the cytosol. Once the bacteria escape into the cytosol, a new arsenal is put into motion by the host cells through activation of caspase-1 and caspase-3, geared toward pyroptosis and apoptosis of the infected cells. Concomitantly, there is activation of NF-κB geared toward triggering pro-survival signals and the induction of proinflammatory cytokines. Intuitively, F. tularensis has devised different strategies to counteract the innate host defense mechanisms. These include inhibition of components of the host defense mechanism and hijacking the cells own defense system and other signaling pathways through bacterial effectors that are likely exploited through a type VI-like secretion system. For example, F. tularensis co-opts the host cell NF-κB transcription factor, which is used to activate proinflammatory cytokines, to induce the expression of anti-apoptotic genes to maintain cell viability. Similarly, F. tularensis co-opts the host cell Ras signaling pathway to inhibit caspase-3-induced apoptosis. Finally, F. tularensis utilizes the host cell ubiquitin-dependent proteasome degradation system to degrade MHC class II molecules on activated macrophages to inhibit antigen presentation to effector T cells.

Many virulence factors have been identified that are required for bacterial escape and replication within the cytosol. Although some of these are involved in known pathways, majority of these have no known functions. The roles of some of these factors in the virulence mechanisms exhibited by F. tularensis are beginning to be defined but the functions are largely unknown. Cytosolic F. tularensis activates PKC leading to activation of Ras and inhibition of apoptosis. MigR regulates genes that are important of phagosome biogenesis. Identifying and characterizing MigR-regulated genes will lead to an understanding of how F. tularensis arrest phagosome maturation. Delineating how MviN, FTT0584, and FTT0748 inhibit caspase-1 activity will shed light on how F. tularensis modulate pyroptosis and proinflammatory cytokine induction. Since NF-κB is required for both cytokine induction and inhibition of apoptosis, its activation must be tightly controlled. It will be interesting to identify the bacterial factors important for activation of Ras and diverson of NF-κB to the expression of anti-apoptotic effectors. It is not known how F. tularensis tilts the balance of power between Akt and SHIP toward SHIP activation and bacterial escape and the mechanism by which this is achieved. Unlike L. monocytogenes, no hemolysin-like molecule have been identified in F. tularensis and there is contradictory data on role of AcpA in escape, which may be partly due to the studies being done using different species of Francisella and different sources of macrophages. It will be interesting to know if SHIP does not only inhibit cytokine activation but also activate a host cell factor that leads to disruption of the phagosome membrane and escape of the bacteria. It will be interesting to determine how F. tularensis modulate the ubiquitin ligase in activated macrophages leading to degradation of MHC II molecules and evasion of adaptive immunity. There is little doubt that F. tularensis employs various strategies to modulate cellular processes that have evolved to degrade invading microbes in addition to evasion of various innate and adaptive immune processes to inflict disease in the mammalian host. It is just as interesting to uncover the molecular and cellular bases of the interaction of F. tularensis with the arthropod vector and its role in pathogenic evolution and infection of the mammalian host. It is likely that the pathogen exploits conserved eukaryotic processes to infect evolutionarily distant hosts as well as processes unique to the infection of mammals.
within the cytosol.

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