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Interaction between Intracellular Bacterial Pathogens and Host Cell Mitochondria

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INTRODUCTION

Mitochondria are dynamic organelles, which are fundamental to eukaryotic cell function. They originated from an endosymbiotic alphaproteobacterium of the genus *Rickettsia*, which was internalized by the ancestor of all eukaryotes (1). Consistent with this endosymbiotic event, mitochondria are surrounded by a double membrane and still share molecular and morphological features with prokaryotic cells, such as the ability to create energy in the form of ATP through aerobic respiration. To do so, mitochondria oxidize nutrients in a process termed oxidative phosphorylation, which involves the creation and harnessing of a membrane potential across the inner mitochondrial membrane, resulting in ATP synthesis.

Apart from energy production, mitochondria carry out essential steps of heme, iron-sulfur cluster, and amino acid biosynthesis as well as fatty acid oxidation and play an important role in calcium homeostasis and cell-autonomous innate immunity (2). In this context, mitochondria display antimicrobial activity through reactive oxygen species (ROS) production and through signaling. Mitochondrial innate immune signaling is mediated by the mitochondrial antiviral signaling protein (MAVS) and results in an interferon response (2, 3). Importantly, mitochondria also play a key role in apoptosis, as the intrinsic apoptosis pathway converges on mitochondrial outer membrane permeabilization (MOMP), which represents a point of no return. Mitochondrion-mediated apoptosis is highly regulated by members of the B cell lymphoma 2 (Bcl-2) protein family; proapoptotic BH3-only proteins are activated by intracellular stress signals, overcome the inhibitory effect of antiapoptotic Bcl-2 proteins, and enhance recruitment of Bcl2-associated X protein (Bax) and Bcl-2 antagonist or killer (Bak) to the mitochondrial outer membrane. There, Bax and Bak oligomerization results in MOMP and allows the release of cytochrome *c*, second mitochondrion-derived activator of caspases (SMAC), and Omi, promoting caspase activation and apoptosis (4).

Along with innate immune signaling and apoptosis, the highly dynamic morphology of mitochondria is one of the characteristics of the organelle that clearly differentiate it from most bacteria. Mitochondrial morphology is determined by a steady-state balance between the opposing events of fusion and fission, which are mediated by a set of dynamin-related GTPases. Mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) coordinate outer membrane fusion by homo- and heterotypic interactions, while optic atrophy 1 (Opa1) mediates fusion of the inner membrane. The current model for mitochondrial fission involves initial mitochondrial constriction through the endoplasmic reticulum (ER) and actin, followed by recruitment of dynamin-related protein 1 (Drp1) to its receptors on the mitochondrial outer membrane. There, Drp1 oligomerizes to form ring-like structures and mediates GTP-dependent mitochondrial fission in conjunction with dynamin 2 (5). Interestingly, Drp1-dependent mitochondrial fission is observed during apoptosis but is not strictly required for its progression (4). Depending on the cell type and functional status of mitochondria, they can adapt their morphology according to cellular energy demands (6) and move along cytoskeletal tracks with the help of molecular motors (7).

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Owing to their central role in multiple cellular processes, mitochondria are an attractive target for pathogens. Modulation of mitochondrial functions can be advantageous for bacteria in terms of access to nutrients and/or evasion of the humoral immune system. Here, we explore the relationship between intracellular bacteria and host cell mitochondria, primarily focusing on the effect of the bacteria on mitochondrial morphology and manipulation of host cell death. Manipulation of cell death allows the bacteria to either preserve their intracellular niche by enhancing survival of the host cell or favor dissemination by inducing host cell death. We present examples of both cytosolic and intravacuolar pathogenic bacteria, including *Listeria monocytogenes*, *Shigella flexneri*, *Rickettsia* spp., *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Salmonella enterica*, *Chlamydia* spp., and *Ehrlichia chaffeensis*. While cytosolic bacteria are able to directly interact with mitochondria and other organelles, intravacuolar pathogens are confined within a membrane-enclosed vacuole and employ specialized secretion systems to introduce effector proteins into the host cell cytoplasm that target mitochondria.

CYTOSOLIC BACTERIA

Listeria monocytogenes

The Gram-positive bacterium *L. monocytogenes* is a facultative intracellular pathogen causing the foodborne disease listeriosis, which mainly and most severely affects immunocompromised individuals. *L. monocytogenes* is capable of invading both phagocytic and nonphagocytic cells and employs the phospholipases PlcA and PlcB and the pore-forming toxin listeriolysin O (LLO) to escape the phagosome (8). Inside the cytosol, *L. monocytogenes* replicates and hijacks the host actin polymerization machinery in order to spread nonlytically to neighboring cells (9). Infection of epithelial cells with *L. monocytogenes* interferes with mitochondrial dynamics and induces a strong and rapid but transient fragmentation of the mitochondrial network at early time points of infection. The fragmentation is specific to virulent *L. monocytogenes*, and the secreted toxin LLO has been identified as the causative factor, but the exact mechanism remains to be elucidated. LLO appears not to localize to mitochondria, but rather oligomerizes and forms pores in the plasma membrane, causing a calcium influx, which is crucial for the induction of mitochondrial fission (10) (Fig. 1). Moreover, the *L. monocytogenes*-induced mitochondrial fragmentation is atypical, as it is independent of Opa1 and Drp1. Indeed, Drp1 dissociates from mitochondria upon infection. On the other hand, the ER and actin, which both have been suggested as regulators of canonical mitochondrial fragmentation, play a role in this type of mitochondrial fission (11).

L. monocytogenes-induced mitochondrial fragmentation is not associated with apoptosis, as classical apoptotic markers such as cytochrome *c* release and Bax recruitment to mitochondria are absent. Nevertheless, LLO impacts mitochondrial function, since it causes a dissipation of the mitochondrial membrane potential as well as a drop in respiration activity and cellular ATP levels (10). Whether mitochondrial fragmentation directly impacts the host cell metabolic switch to glycolysis (12) remains speculative. As both the mitochondrial network morphology and ATP level recover within a few hours, mitochondria seem not to be terminally damaged upon infection. Interestingly, mitochondrial dynamics plays an important role in *L. monocytogenes* infection. It was shown that treatment with small interfering RNA favoring mitochondrial fusion augments the infection efficiency, whereas cells with fragmented mitochondria are less susceptible to *L. monocytogenes* infection. Based on these observations, it was proposed that *L. monocytogenes* targets mitochondria to temporarily impair mitochondrial functions in order to establish its replication niche (10). Subsequent studies showed that one of the mitochondrial functions, i.e., cell-autonomous innate immune signaling through MAVS, is not active during *L. monocytogenes* infection, and innate immune signaling is rather mediated by peroxisome-localized MAVS (13).

Shigella flexneri

S. flexneri is a Gram-negative bacterium which causes shigellosis, an inflammatory disease of the colon leading to tissue destruction, and a leading cause of diarrhea in the developing world. After crossing the colonic epithelium, the facultative intracellular pathogen infects both myeloid immune cells and intestinal epithelial cells. *S. flexneri* injects secreted effectors into the host cell by its type III secretion system (T3SS) to induce membrane ruffling, resulting in enterocyte invasion. The bacterium then rapidly escapes from the phagosome and proliferates in the cytosol, where it employs the host cell actin machinery for intracellular motility as well as for cell-to-cell spread (14). Interestingly, mitochondria were observed at bacterial invasion sites and appear to be entrapped in an actin meshwork induced by the bacterium (15). The authors of the study proposed a model in which an increase in mitochondrial calcium concentration would activate mitochondrial ATP production to locally provide ATP for further actin polymerization (15).

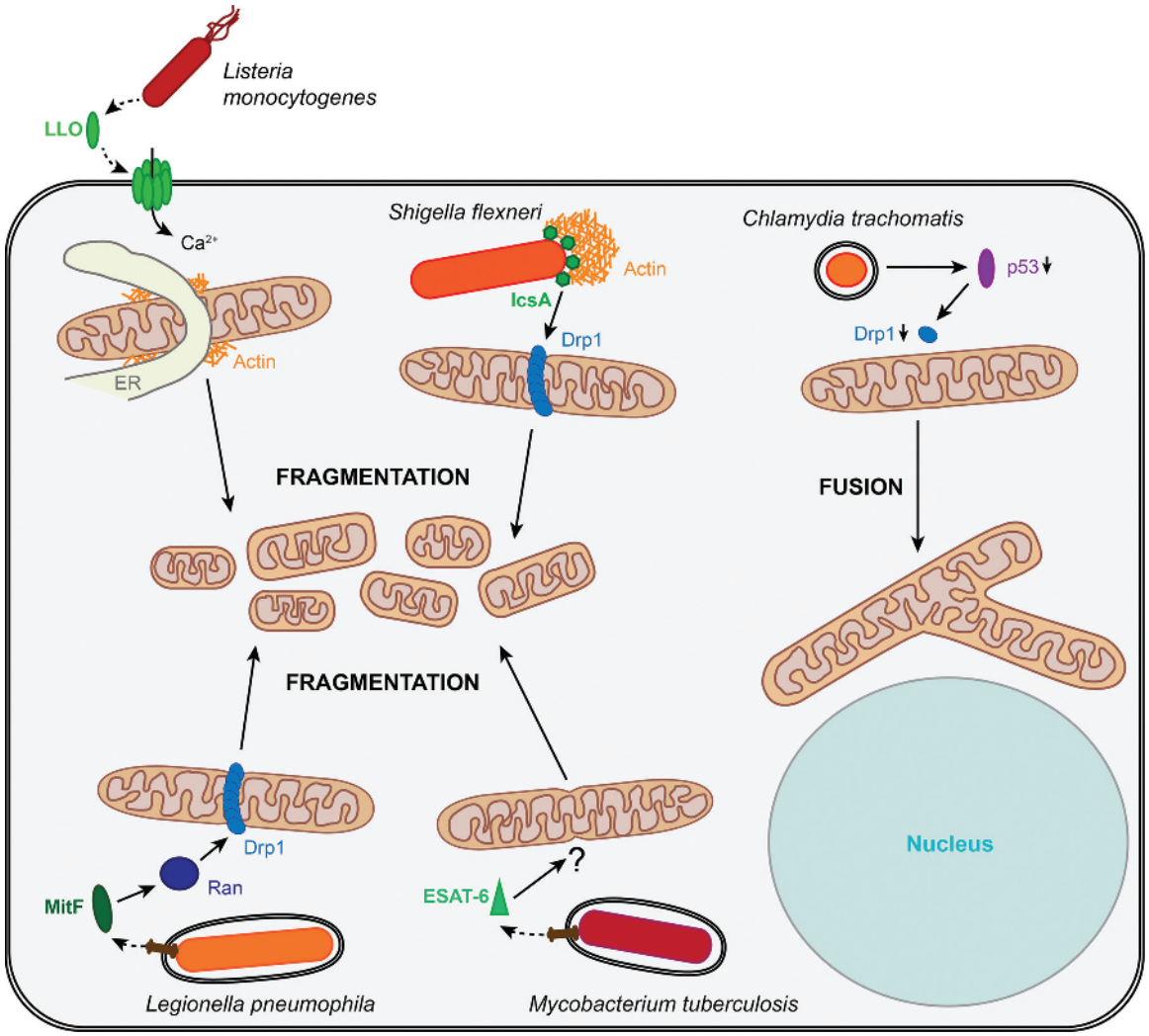

Figure 1 Strategies of intracellular bacteria to interfere with mitochondrial morphology. In epithelial cells, the secreted *L. monocytogenes* toxin LLO induces rapid mitochondrial fragmentation by pore formation in the plasma membrane, enabling calcium influx. Independent of Drp1 and Opa1, *L. monocytogenes*-induced mitochondrial fragmentation is of an atypical type; however, the ER and actin appear to play a regulatory role. The *S. flexneri* surface protein IcsA leads to Drp1-dependent mitochondrial fission in epithelial cells. Infecting macrophages, *L. pneumophila* induces mitochondrial fragmentation in macrophages by the secreted MitF, which activates Ran GTPase and triggers Drp1 recruitment. Infection of epithelial cells with *M. tuberculosis* leads to mitochondrial fission, induced by the bacterial pore-forming toxin ESAT-6. In contrast to the other bacteria shown here, *C. trachomatis* stabilizes the mitochondrial network; downregulating p53, the bacterium inhibits Drp1 expression and recruitment and prevents mitochondrial fragmentation.

At later time points of infection (3.5 h), *S. flexneri* infection induced mitochondrial fission, which was dependent on Drp1 (16). Another study reported local mitochondrial fragmentation in the context of counteracting host cell defense through septin cages. Septin cages have been described to reduce infection by actin-polymerizing bacteria, targeting them to autophagosomes, thus limiting both their motility and dissemination (17). Whereas mitochondrial recruitment to *S. flexneri* contributes to the formation of septin cages, local mitochondrial fission induced by the bacterial surface protein IcsA (Fig. 1) has been shown to prevent septin cage formation (18).

At the functional level, *S. flexneri* has been found to cause a dissipation of the mitochondrial membrane potential and a decrease in the cellular ATP levels by around 50%, correlating with *S. flexneri*-induced necrotic cell death in both epithelial cells (19) and macrophages (20). In epithelial cells, necrosis is counterbalanced by the bacterium, inducing Nod1 signaling. This signaling activates the prosurvival nuclear factor κB (NF-κB) signaling pathway, which in turn inhibits the BNIP3-CypD-dependent opening of the mitochondrial permeability transition pore, a crucial player in the induction of necrosis (19).

Rickettsia spp.

Rickettsia species are Gram-negative obligate intracellular bacteria and are further classified into two major antigenically defined groups, the typhus group and the spotted fever group. *Rickettsia prowazekii* represents the prototype of the typhus group and is transmitted by lice to humans, causing epidemic typhus. *Rickettsia rickettsii* and *Rickettsia conorii* belong to the spotted fever group and are the causative agents of Rocky Mountain spotted fever and Mediterranean spotted fever, respectively. *R. prowazekii*, *R. rickettsii*, and *R. conorii* preferentially infect vascular endothelial cells lining small and medium-sized blood vessels. After entering the host cell via induced phagocytosis, the bacteria rapidly escape the phagosome and replicate in the cytoplasm (21, 22). It has been shown that *R. prowazekii* and several spotted fever group species import the host mitochondrial protein VDAC1 (voltage-dependent anion-selective channel 1), which localizes to contact sites between inner and outer bacterial membranes and appears to be functional. The authors suggested that a primitive protein import mechanism hijacking mitochondrial proteins underlies the obligate endosymbiotic lifestyle of rickettsiae (23, 24). In order to maintain the endothelial cell as a bacterial replication niche, *R. rickettsii* suppresses apoptosis by activation of the prosurvival protein NF-κB (25). By regulating levels and localization of pro- and antiapoptotic Bcl2-family proteins, *R. rickettsii* furthermore maintains mitochondrial integrity and inhibits the activation of caspases 8 and 9 (26, 27).

VACUOLAR BACTERIA

Legionella pneumophila

L. pneumophila is a facultative intracellular bacterium infecting a wide range of hosts, ranging from amoebae to humans. In humans, *L. pneumophila* replicates in alveolar macrophages and causes Legionnaires' disease, a serious pulmonary infection. Inside the host cell, the Gram-negative bacterium resides inside an *L. pneumophila*-containing vacuole (LCV) and is able to evade fusion with the endosome. To manipulate host functions and allow bacterial replication, *L. pneumophila* injects more than 200 bacterial proteins into host cells via a type IV secretion system (T4SS) (28). It has been reported that 30% of LCVs associate with mitochondria as early as 15 min after infection, and the proportion increases to up to 65% after 1 h of infection (29). A close proximity of mitochondria to LCVs was also shown in *L. pneumophila*-infected amoebae (30).

In contrast to these data, more recent time-lapse imaging analyses failed to highlight a stable association of mitochondria with LCVs in *Drosophila* S2 cells (31) or in human primary macrophages (32). The latter study identified transient and highly dynamic mitochondrion-LCV contacts with virulent or avirulent (T4SS-deficient) strains (32). Escoll and colleagues further demonstrated that *L. pneumophila* induces mitochondrial fragmentation without induction of host cell apoptosis at 6 h postinfection (32). Indeed, previous studies had already proposed that *L. pneumophila* inhibits apoptosis, as the number of apoptotic cells remains stable despite effective bacterial replication (33). The secreted bacterial effectors SdhA (34) and SidF were later shown to prevent apoptosis. While the mode of SdhA action remains elusive, SidF mediates apoptotic resistance by specifically interacting with the proapoptotic Bcl-2 proteins BNIP3 and Bcl-rambo (35).

The bacterial factor inducing mitochondrial fragmentation upon *L. pneumophila* infection has been identified and termed mitochondrial fragmentation factor (MitF). MitF is a T4SS-secreted effector that was shown to promote Drp1-dependent mitochondrial fragmentation through a yet-to-be-discovered mechanism involving the nuclear transport factors Ran and Ran-binding protein B2 (RanBP2) as host targets of MitF (Fig. 1). Furthermore, *L. pneumophila*-induced mitochondrial fragmentation correlates with an alteration of the host cell energy metabolism by impairing mitochondrial respiration, leading to reduced mitochondrial ATP production and a decrease in ATP levels. Simultaneously, host cell glycolysis is upregulated and was shown to favor *L. pneumophila* intracellular replication, while mitochondrial respiration appears to be dispensable (32). In line with these findings, it was reported that the secreted *L. pneumophila* mitochondrial carrier protein LncP is not crucial for bacterial proliferation, even though it is targeted to the mitochondrial inner membrane, where it transports ATP from the matrix to the intermembrane space (36).

Mycobacterium tuberculosis

M. tuberculosis is the causative agent of tuberculosis, an infectious disease affecting approximately one-third of the world's population asymptomatically and leading to 1.8 million deaths annually (37). A facultative intracellular bacterium, *M. tuberculosis* colonizes primarily human monocytes and macrophages, where it replicates in a specialized phagosomal compartment. By preventing the fusion of the phagosome with lysosomes and inhibiting phagosomal acidification, *M. tuberculosis* preserves its intracellular niche and replicates. Eventually, *M. tuberculosis* induces necrosis of the host cell in order to spread to neighboring cells. The initiation of apoptosis is therefore considered a defense strategy of host cells to restrict *M. tuberculosis* spreading, as apoptotic cells maintain their contents inside and are cleared by phagocytes (38). In agreement with this hypothesis, infection of macrophages with an avirulent *M. tuberculosis* strain induces apoptosis at a higher level than infection with a virulent strain (39). Consistently, infection with virulent *M. tuberculosis* has been shown to upregulate antiapoptotic proteins such as Bcl-2 (40) and myeloid cell leukemia 1 (Mcl-1) (41). Regarding the interaction of *M. tuberculosis* and mitochondria, recent studies focused on mitochondrial implication in cell death modulation. Chen and colleagues correlated the primarily induced cell death in macrophages with mitochondrial membrane perturbations. While both virulent and avirulent strains lead to transient MOMP and cytochrome *c* release, only the virulent strain causes significantly more mitochondrial permeability transition at early infection time points (6 h). As a consequence, the virulent strain rapidly triggers necrosis, whereas the avirulent strain induces apoptosis only 48 h after infection (42).

Metabolomic profiling on aqueous tissue extracts suggested that *M. tuberculosis* infection in mice leads to an upregulation of host cell glycolysis (80), consistent with previous findings reporting mitochondrial damage (42, 43). In contrast, Jamwal and colleagues observed an increased mitochondrial membrane potential in cells infected with the virulent strain (44). Furthermore, the authors analyzed the mitochondrial response to infection at the proteomic level, revealing infection-induced upregulation of the mitochondrial protein VDAC2 (44), which appears to prevent apoptosis by keeping the proapoptotic protein Bak inactive (45).

Studies on the effect of *M. tuberculosis* infection on mitochondrial morphology are controversial. While one study described mitochondrial swelling and a reduction of the mitochondrial matrix density in J744 macrophages infected with virulent and avirulent *M. tuberculosis* (43), another study reported different mitochondrial phenotypes and activities depending on the virulence of the strain. Upon infection with virulent H37Rv, monocytic THP-1 cells display elongated mitochondria with an increased electron density and augmented activity; in contrast, cells infected with the avirulent H37Ra strain appear to have less electron-dense mitochondria, which are considered nonfunctional (44). Infection of alveolar epithelial cells with a virulent strain caused mitochondrial fragmentation and aggregation in the perinuclear region at late time points of infection (48 h). The pore-forming toxin ESAT-6 has been proposed as the virulence factor responsible for this effect, as its absence prevents mitochondrial fragmentation and aggregation (46) (Fig. 1).

Salmonella enterica

S. enterica is a foodborne Gram-negative facultative intracellular bacterium causing gastroenteritis. To establish infection, *S. enterica* manipulates host cell functions through a plethora of secreted effector proteins. These effectors are secreted through two T3SSs and contribute to the very early steps of infection by inducing membrane ruffling, which mediates pathogen uptake into nonphagocytic host cells, where *S. enterica* then persists in a vacuole (47). In order to ensure bacterial survival and replication within epithelial cells, *S. enterica* remodels the vacuole to prevent its fusion with lysosomes (48) and inhibits host cell apoptosis. In recent years, *Salmonella* outer protein B (SopB) and fimbrial protein subunit A (FimA) have been identified as two secreted bacterial effector proteins which interfere with mitochondrial functions such as ROS production and apoptosis. SopB binds to cytosolic tumor necrosis factor receptor-associated factor 6 (TRAF6) and delays its mitochondrial recruitment, which causes decreased generation of mitochondrial ROS. In addition to this, Bax translocation to mitochondria and pore formation are inhibited, and induction of apoptosis by cytochrome *c* release is prevented (49). On the other hand, the soluble form of the pilus protein FimA targets mitochondria, where it binds to the outer mitochondrial membrane protein VDAC1. This results in a tight association between VDAC1 and the mitochondrial hexokinases, suppressing the integration of the pore-forming protein Bax into the outer mitochondrial membrane and the release of cytochrome *c* (50). Two mechanisms thus converge to prevent cytochrome *c* release from mitochondria in *S. enterica*-infected cells. Another effector, called *Salmonella* outer protein A (SopA), has been reported to localize to mitochondria (51). SopA was lately described as interacting with two host E3 ubiquitin ligases, TRIM56 and TRIM65, inducing an innate immune

response involving MAVS activation and characterized by enhanced interferon beta signaling (52).

In contrast to *S. enterica* infection in epithelial cells, *S. enterica* infection induces programmed cell death in macrophages. In this context, Hernandez and colleagues proposed that the effector SipB localizes to mitochondria and disrupts mitochondrial morphology, causing swelling and loss of cristae integrity, triggering mitochondrial disruption and resulting in a caspase 1-independent and autophagy-mediated cell death (53).

Chlamydia spp.

The genus *Chlamydia* comprises three Gram-negative bacterial species, which are pathogenic to humans: *Chlamydia trachomatis*, *C. pneumoniae*, and *C. psittaci*. *C. trachomatis* is one of the most common sexually transmitted bacteria and causes trachoma, a severe eye infection that can lead to blindness. *C. pneumoniae* and *C. psittaci* are associated with respiratory infections such as pneumonia. The obligate intracellular *Chlamydia* species exhibit a characteristic biphasic life cycle with two distinct developmental forms. The infectious elementary bodies (EBs) attach to epithelial cells and are taken up by phagocytosis. Inside host cells, EBs reside inside membrane-bound inclusions, where they differentiate into metabolically active reticulate bodies (RBs). RBs proliferate by binary fission and undergo maturation to again form infectious EBs, which are released upon host cell lysis in order to infect new cells (54). Several *Chlamydia* species have been shown to prevent host cell apoptosis by acting on mitochondria (55). Upon *C. trachomatis* and *C. pneumoniae* infection, the chlamydial protease-like activity factor induces the degradation of proapoptotic BH3-only proteins, such as Bim, Puma, and Bad, thereby suppressing cytochrome *c* release from mitochondria and mediating cellular resistance to apoptosis (56, 57).

In terms of energy supply, *Chlamydia* species have been described as "energy parasites," as they depend on the import of host cell ATP and metabolites (58, 59). In agreement with this hypothesis, chlamydial infections influence localization and morphology of mitochondria, presumably to obtain ATP and metabolites. By employing electron microscopy, Masumoto identified a tight association of *C. psittaci* with mitochondria, supporting the former biochemical observations. Mitochondrial association occurred approximately 12 h postinfection, at the time when RBs start to replicate (60).

Interestingly, although all *Chlamydia* species possess genes encoding ATP transporters, the recruitment of mitochondria to the inclusion is unique to *C. psittaci* and was not observed for *C. trachomatis* or *C. pneumoniae* (61). Instead, *C. trachomatis* stabilizes the mitochondrial network upon infection-induced stress in order to preserve the mitochondrial ATP production capacity. To do so, *C. trachomatis* prevents Drp1-mediated mitochondrial fragmentation by downregulating p53 (62), a known regulator of Drp1 expression (63) (Fig. 1), and apoptosis. Mitochondrial morphology affects *C. trachomatis* infection, and cells with fragmented mitochondria display decreased infection levels (62). Liang and colleagues demonstrated a dependency of *C. trachomatis* EBs on mitochondrial energy production in epithelial cells; however, the authors also showed that later during infection, *C. trachomatis* RBs rely only to a limited extent on mitochondrial ATP and employ a sodium gradient to produce energy (64). In contrast to *C. trachomatis* infection, *C. pneumoniae* infection causes mitochondrial dysfunction characterized by mitochondrial hyperpolarization, increased ROS generation, and the induction of a metabolic switch to host cell glycolysis. Consistently, impairment of mitochondrial function enhances growth of *C. pneumoniae* inclusions (65).

Ehrlichia chaffeensis

E. chaffeensis is an obligate intracellular bacterium which causes the tick-borne disease human monocytic ehrlichiosis. This Gram-negative pathogen infects and proliferates inside monocytes and macrophages, where it resides inside vacuoles and forms characteristic mulberry-like bacterial aggregates, which are referred to as morulae. The first evidence that mitochondria play an important role for *E. chaffeensis* infection was reported by Popov and colleagues; they observed mitochondria closely associated with bacteria-containing morulae in infected macrophages (67). Functional studies of the interaction revealed that *E. chaffeensis* infection does not cause a change in the mitochondrial membrane potential; however, it reduces mitochondrial DNA synthesis and transcription of mitochondrial genes (68). These observations suggest that *E. chaffeensis* inhibits mitochondrial activity. Surprisingly, a screen in *Drosophila melanogaster* identified seven mitochondrion-associated genes whose mutation results in increased resistance to infection (69). A key process in *E. chaffeensis* pathogenicity is the secretion of effector proteins, which allow evasion of bacterial killing by preventing lysosomal degradation and inhibiting apoptosis, thereby preserving the bacterial replication niche (66). For example, *E. chaffeensis* secretes the bacterial effector ECH0825, which localizes to mitochondria and inhibits Bax-induced apoptosis. It has been furthermore proposed that ECH0825 prevents ROS-induced cellular stress and apoptosis by upregulating

mitochondrial manganese superoxide dismutase (70). Transcriptional profiling of cells infected with *E. chaffeensis* revealed the induction of the antiapoptotic protein NF-κB and of the Bcl2 proteins Bcl2A1 and Mcl-1 as well as repression of the proapoptotic proteins Bik and BNIP3 (71).

CONCLUSIONS

In this chapter, we summarize several findings illustrating the importance of mitochondria during bacterial infection, involving the manipulation of mitochondrial morphology and function or the recruitment of mitochondria to the infection site. While viruses induce either

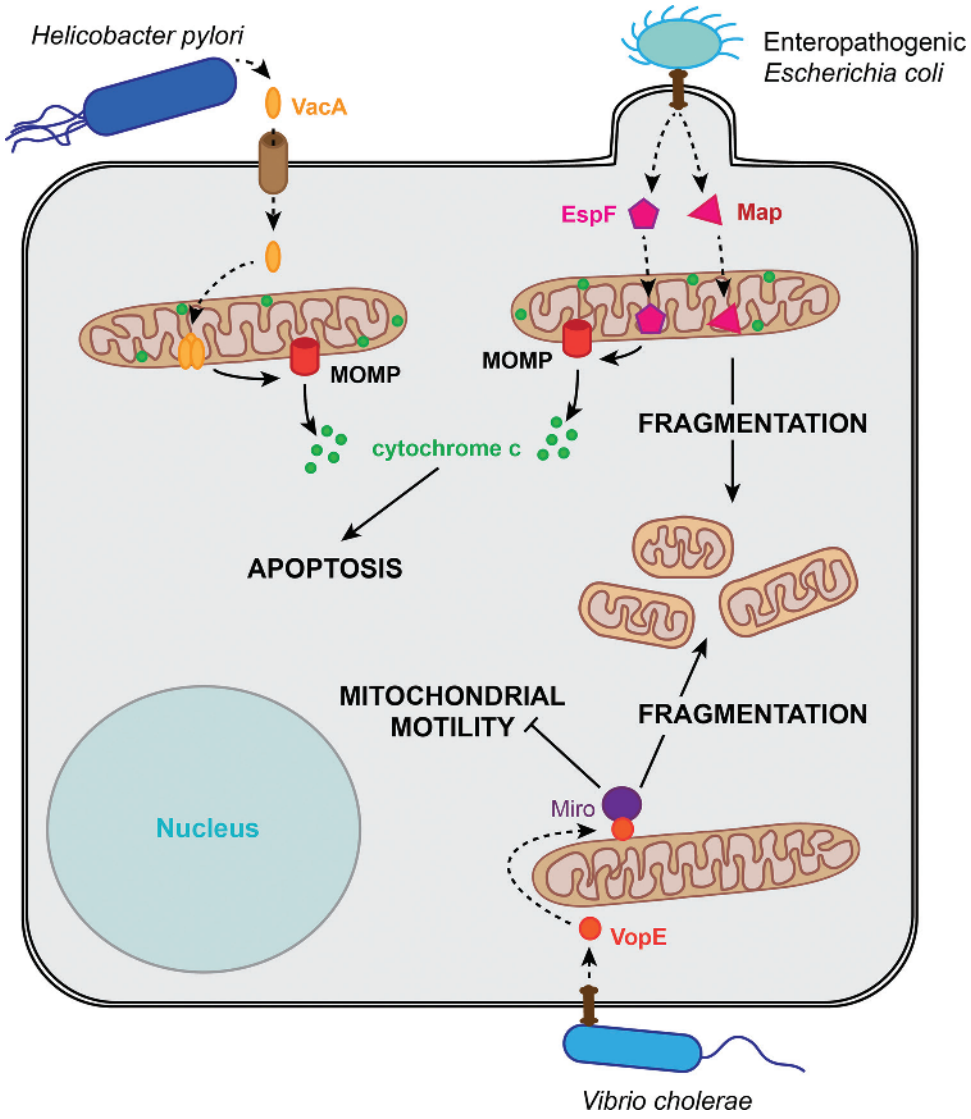

Figure 2 Relationship between extracellular bacteria and host cell mitochondria. The extracellular bacterium *H. pylori* secretes VacA, a pore-forming toxin, which localizes to the mitochondrial inner membrane and induces MOMP, resulting in apoptosis. EPEC interferes with mitochondrial morphology and function by the secretion of the effector proteins Map and EspF. Both proteins localize to the mitochondrial matrix, where Map leads to mitochondrial fragmentation and EspF induces MOMP and subsequent apoptosis. *V. cholerae* secreted VopE is a GTPase-activating protein that inactivates Miro at mitochondria, causing mitochondrial fragmentation and inhibiting kinesin-dependent mitochondrial motility. Movement is represented by dashed arrows, while solid arrows indicate induction.

mitochondrial fission or fusion (72), to date, bacterial infections seem to mainly induce mitochondrial fission. Although such fission appears to proceed either via the classical, Drp1-dependent fission pathway (*S. flexneri* and *L. pneumophila*) or through an atypical, Drp1-independent pathway (*L. monocytogenes*), fragmentation of the mitochondrial network may represent a common bacterial strategy to impact different mitochondrial functions, or a cellular stress response. Strikingly, mitochondrial morphology can impact the success of infection; cells with fragmented mitochondria display a reduced rate of infection by both *L. monocytogenes* and *C. trachomatis*.

Several bacteria use a similar mechanism to interfere with mitochondrial morphology and function, relying on secreted bacterial effectors. These effectors induce changes in mitochondrial structure, dynamics, and functionality which allow bacteria to preserve their replicative niche. Many of the bacterial effectors target Bcl-2 family members by modulating their expression and activity in order to suppress apoptosis. Several secreted effectors that affect mitochondrial morphology and function are pore-forming toxins, and pore formation is essential for their effects. While *L. monocytogenes* enables calcium influx through pores in the plasma membrane formed by the secreted toxin LLO (Fig. 1), vacuolating cytotoxin A (VacA) injected by extracellular *Helicobacter pylori* localizes to mitochondria and has been shown to form pores in the inner mitochondrial membrane, inducing a loss of the mitochondrial membrane potential and apoptosis (73, 74) (Fig. 2). In contrast, the exact mechanism by which the pore-forming toxin ESAT-6 from *M. tuberculosis* affects mitochondria remains speculative (Fig. 1). Other secreted bacterial effectors rely on different mechanisms to induce mitochondrial fission: for example, *L. pneumophila* MitF indirectly triggers Drp1 oligomerization to cause mitochondrial fragmentation (Fig. 1).

Several extracellular bacteria also secrete effectors that target mitochondrial dynamics and function through diverse mechanisms. The extracellular bacterium *Vibrio cholerae* secretes a GTPase-activating protein (VopE), which promotes mitochondrial fragmentation and prevents kinesin-dependent mitochondrial motility (Fig. 2). Thereby, VopE inhibits mitochondrial perinuclear clustering and MAVS-dependent innate immune responses (75). Enteropathogenic *Escherichia coli* (EPEC) effector proteins mitochondria-associated protein (Map) and EPEC-secreted protein F (EspF) display yet another way of targeting mitochondria, as they localize to the mitochondrial matrix and act from within (Fig. 2). While both proteins disrupt the mitochondrial membrane potential, EspF has been shown to trigger apoptosis (76), while Map causes mitochondrial fragmentation and might work in an antiapoptotic fashion and control other mitochondrion-regulated cellular processes (77, 78).

Another way by which bacteria affect mitochondrial morphology is through the recruitment of mitochondria. This has been observed for several cytosolic and intravacuolar bacteria, which thereby presumably benefit from mitochondrion-derived ATP or other metabolites. An extreme example of a bacterium that may benefit from mitochondrial functions or metabolites is the alphaproteobacterium "*Candidatus* Midichloria mitochondrii," which invades mitochondria of tick ovarian cells (79).

Bacterial infection can affect not only mitochondrial morphology but also the host cell energy metabolism. In *L. pneumophila* infection, mitochondrial fragmentation correlates with a decrease in mitochondrial respiration. A similar scenario might apply to *L. monocytogenes* infection. Interestingly, although *C. pneumoniae* infection causes mitochondrial hyperpolarization and increased ROS production, it also triggers host cell glycolysis. Reprogramming of the host cell energy metabolism has also been reported for other intracellular bacteria, such as *L. monocytogenes* and *M. tuberculosis*. In contrast, *Francisella tularensis* infection was found to inhibit glycolysis in macrophages (81). However, the link between mitochondria and these infection-induced metabolic changes remains largely unknown.

The complex interactions between bacteria and mitochondria summarized here highlight the importance of this organelle in infection. Future studies will shed more light on the mechanisms by which bacteria affect mitochondria and afford a better understanding of the specific roles that mitochondria play during different stages of bacterial infection.

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