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Control of host mitochondria by bacterial pathogens

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Abstract

Mitochondria control various processes that are integral to cellular and organismal homeostasis, including Ca^{2+} fluxes, bioenergetic metabolism and cell death. Perhaps not surprising, multiple pathogenic bacteria have evolved strategies to subvert mitochondrial functions in support of their survival and dissemination. Here, we discuss non-immunological pathogenic mechanisms that converge on the ability of bacteria to control the mitochondrial compartment of host cells.

Introduction

Despite originating in the context of an endosymbiotic relationship >1.45 billion years ago, modern mitochondria remain at the very center of eukaryotic life as they control a variety of cellular processes that are fundamental for the preservation of both cellular and organismal homeostasis [1,2]. Mitochondria are structurally delimited by two membranes, which delineate an inner mitochondrial matrix and an intermembrane space (IMS). The mitochondrial matrix houses 2-10 copies of a small (approximately 16,568 base pairs in humans) circular genome generally referred to as mitochondrial DNA (mtDNA) as well as (1) a functional machinery for protein synthesis, and (2) numerous enzymes involved in multiple cellular functions (see below). The inner mitochondrial membrane (IMM) forms pronounced invaginations called cristae, resulting in a considerable increase in IMM surface area that is instrumental for the mitochondria to synthesize large amounts of ATP via oxidative phosphorylation (OXPHOS). Moreover, mitochondria are intimately involved in the regulation of intracellular Ca^{2+} fluxes, and contain a refined molecular machinery that precipitates regulated cell death (RCD) via apoptosis or **mitochondrial permeability transition (MPT)**-dependent regulated necrosis [3]. Finally, mitochondria have acquired the ability to emit and decode molecular signals elicited by infection and other stress conditions, constituting one of the first lines of cellular defense against both viral and bacterial pathogens [4]. Thus, various pathogenic organisms that colonize the intracellular microenvironment have evolved strategies to govern the mitochondrial functions of host cells in support of their own survival, replication and dissemination (**Table 1**).

While the mechanisms through which bacterial pathogens inhibit innate immune signaling by host cells in support of immunoevasion have been recently discussed in detail [5] (**Box 1**), a unified and updated overview of the non-immune mitochondrial processes that bacteria

subvert to their own benefit is missing. Here, we critically summarize the ability of pathogenic bacteria to sabotage bioenergetic metabolism, Ca^{2+} fluxes, as well as **mitophagy** and apoptotic cell death by targeting the mitochondria of host cells.

Bioenergetic metabolism

In normoxic conditions, most eukaryotic cells harness glycolysis to convert glucose into pyruvate, which enters the mitochondria to generate acetyl-CoA and reducing equivalents via the **tricarboxylic acid (TCA) cycle**. Such reducing equivalents are then oxidized to produce 30-36 ATP molecules (per glucose molecule) via OXPHOS. Conversely, in hypoxic environments, eukaryotic cells resort to the glycolytic conversion of glucose into lactate (which provides only 2 ATP molecules per glucose molecule, but does not require molecular oxygen as OXPHOS does). In many cases, bacterial infection is accompanied by a global metabolic rewiring from OXPHOS to glycolysis that may be detrimental to invading pathogens [6]. As an example, phagocytic cells exposed to lipopolysaccharides (LPS) experience a robust inhibition of the TCA cycle coupled with the accumulation of some of its intermediates, such as succinate and itaconate, in support of glycolytic metabolism and lactate production [7]. Besides regulating the emission of pro-inflammatory signals by infected macrophages [8], itaconate strongly inhibits bacterial isocitrate lyase, hence limiting the growth of multiple pathogens, such as *Mycobacterium tuberculosis* and *Salmonella enterica* [9]. Of note, efficient OXPHOS is generally associated with mitochondrial elongation [10], suggesting that LPS-dependent inhibition of the TCA cycle may also benefit invading pathogens indirectly, as a consequence of altered mitochondrial dynamics [11] (see below).

Irrespective of this possibility, other pathogens such as *Pseudomonas aeruginosa* can proliferate by harnessing succinate, which accumulates in the airway as a result of impaired mitochondrial activity driven by CF transmembrane conductance regulator (*CFTR*) mutations [12]. Similarly, *Brucella abortus* disrupts mitochondrial metabolism and OXPHOS in host cells to favor glycolysis, as it can utilize lactate for proliferating [13]. While a similar trophic pathway has been proposed for *M. tuberculosis* [14], accurate metabolic assessments suggest

that *M. tuberculosis* does not shift host cell metabolism towards glycolysis, but instead establishes an ATP-depleted quiescent state (*i.e.*, suppressed OXPHOS coupled with low glycolytic rates) and harnesses fatty acid intake for survival [15]. This bioenergetic rewiring appears to be sustained by the production of ketone bodies coupled to the accumulation of lipid droplets in infected cells [16], leading to the acquisition of the pathognomonic "foamy phenotype" associated classically with *M. tuberculosis* infection.

Intracellular bacteria such as *Chlamydia trachomatis* actively boost OXPHOS, not only as they favor mitochondrial fusion [17], but also as they ensure elevated glucose intake from the extracellular microenvironment [18], ultimately supporting bacterial proliferation as a consequence of abundant ATP production. Interestingly, such an effect may originate from Toll-like receptor 2 (TLR2) activation by bacterial lipopeptides, which appear to sustain OXPHOS in the context of upregulated glycolysis. A concomitant increase in OXPHOS and glycolysis has also been observed also upon *Staphylococcus aureus* infection [19], in the absence of major changes in mitochondrial dynamics (at least in the early phases of infection) [20]. However, such a response does not only favor the survival of invading pathogens as a result of high ATP levels, but also supports innate immune signaling in both immune (*i.e.*, monocytes) [19] and non-immune (*i.e.*, keratinocytes) cells [21].

Taken together, these observations exemplify the co-evolution of bacterial vs. host (immune) metabolic responses, indicating as invading pathogens rapidly adapt to bionergetic alterations to their own survival (**Fig. 1**).

Ca^{2+} fluxes

Alterations of Ca^{2+} signalling frequently contribute to bacterial pathogenicity, as demonstrated by the fact that numerous bacterial toxins evoke intracellular Ca^{2+} oscillations [22]. While increased cytosolic Ca^{2+} levels are normally a consequence of influx from the extracellular milieu or opening of reticular stores, Ca^{2+} ions cross the outer mitochondrial membrane (OMM) through the voltage-dependent anion channels (VDACs) and accumulate at large amounts inside the mitochondrial matrix due to (1) the physical proximity of the mitochondria to the endoplasmic reticulum (ER); (2) the negative electrochemical potential established across the IMM, which acts as a driving force for Ca^{2+} entry; and (3) the presence of a regulated Ca^{2+}-permeable channel, the mitochondrial calcium uniporter (MCU) complex [23,24].

Bacteria-driven cytosolic Ca^{2+} elevations have been associated with RCD via MPT-driven necrosis downstream of mitochondrial Ca^{2+} overload and consequent opening of the **permeability transition pore complex (PTPC)** (see below) [25]. Both *S. aureus* and the atypical fish pathogen *Mycobacterium fortuitum* appear to proficiently initiate this cascade of events, but while the latter has been suggested to drive mitochondrial Ca^{2+} overload by favoring the assembly of **mitochondria-associated ER membranes (MAMs)**, the implication of MAMs in mitochondrial Ca^{2+} accumulation elicited by *S. aureus* remains to be established [26,27]. *Streptococcus pneumoniae* also causes massive mitochondrial Ca^{2+} entry and collapse of the electrochemical gradient, potentially leading to PTPC activation [28]. However, rather than promoting RCD, mitochondrial Ca^{2+} overload in cells infected by *S. pneumoniae* appears to drive innate immune signaling via the release of mtDNA into the cytosol [28-30], pointing to the involvement of hitherto unidentified RCD-delaying factors for optimal *S. pneumoniae* pathogenicity (see below). Indeed, while an excessively rapid

activation of RCD in host cells (prior to pathogen replication) is detrimental to invading bacteria, late RCD (upon pathogen replication) may be advantageous as it favors dissemination. In line with this notion, early signs of mitochondrial dysfunction have been documented upon *Shigella flexneri* infection, partially linked to mitochondrial Ca^{2+} elevations and PTPC opening [31]. However, *S. flexneri* actively prevents premature RCD through the type 3 secretion system (T3SS) effector IpgD, which inhibits Ca^{2+} release from the ER by targeting inositol 1,4,5 trisphosphate receptor (IP_3R) channels [32,33].

Altered mitochondrial Ca^{2+} homeostasis also supports the pathogenicity of various bacteria irrespective of active RCD control [34]. Indeed, moderate increases in mitochondrial Ca^{2+} levels promote bioenergetic metabolism including ATP production [23,24], which may reasonably sustain bacterial growth during prolonged infection. In line with this notion, shortly after infection, *Listeria monocytogenes* drives listeriolisin O (LLO)-dependent cytosolic Ca^{2+} elevations from both extracellular and intracellular sources [35], rapidly evolving in transient mitochondrial Ca^{2+} accumulation via MCU [36] and acetyl-CoA production via the Ca^{2+}-sensitive pyruvate dehydrogenase (PDH) complex [37]. In turn, accumulating acetyl-CoA favors the acetylation of rubicon autophagy regulator (RUBCN), a key regulator of a non-canonical form of autophagy with antibacterial effects termed **LC3-associated phagocytosis (LAP)** [38,39]. Importantly, abolition of mitochondrial Ca^{2+} uptake in myeloid cells via *MCU* deletion improves LAP and bacterial neutralization, demonstrating that *L. monocytogenes* safeguards its intracellular growth in a Ca^{2+}-dependent manner [36].

Taken together, these observations highlight various mechanisms of pathogenicity involving the ability of invading bacteria to control mitochondrial Ca^{2+} fluxes in host cells. This can be achieved by altering the composition of the MCU complex, which comprises both channel-forming and regulatory subunits [40], or by targeting mitochondrial Ca^{2+} efflux systems [41].

In the case of *L. monocytogenes*, increased levels of the MCU regulator mitochondrial calcium uptake 1 (MICU1) [42] appear to enable efficient mitochondrial Ca^{2+} entry upon release from reticular stores, achieving not only a boost in mitochondrial metabolism but also a protection from excessive cytosolic Ca^{2+} levels that reflects the ability of MICU1 to regulate the Ca^{2+} threshold for MCU opening [43]. Mitochondrial Ca^{2+} elevations could also be obtained by altering the activity of solute carrier family 8 member B1 (SLC8B1, best known as NCLX) [42], which is regulated at post-transcriptional level [44]. However, to the best of our knowledge, no invading pathogen has been formally demonstrated to control mitochondrial Ca^{2+} homeostasis in host cells via NCLX. Finally, mitochondrial Ca^{2+} fluxes can be elicited by altering the IP_3R-dependent coupling of the ER and mitochondria at MAMs. This mechanism, which is also expected to prevent autophagy-dependent pathogen clearance [45], has been documented in cells infected by *C. trachomatis*, which can activate IP_3Rs via the inclusion membrane protein MrcA [46].

In summary, the key role of mitochondrial Ca^{2+} at the interface between bioenergetic metabolism and RCD regulation [47] stands out as an ideal target for manipulation by bacterial pathogens at different stages of infection (**Figure 2**). In turn, counteracting the strategies that bacteria have evolved to control the amplitude and kinetics of mitochondrial Ca^{2+} fluxes may represent a promising strategy for the development of novel antimicrobials.

Mitochondrial dynamics

Mitochondria undergo continuous **fusion** and **fission** events, which are tightly regulated at the molecular level. While mitochondrial elongation is mainly promoted by mitofusins (MFNs) at the OMM and OPA1 mitochondrial dynamin like GTPase (OPA1) at the IMM, fission is generally initiated by the translocation of the cytosolic factor, dynamin 1 like (DNM1L, best known as DRP1) to the OMM, where it marks the site of mitochondrial division. Multiple

bacterial species remodel the mitochondrial network, generally as they promote fission in support of RCD during the late infection phases [5]. Indeed, the pro-apoptotic factor BCL2 associated X, apoptosis regulator (BAX) and BCL2 antagonist/killer 1 (BAK1) [48] also recruit DRP1 to the OMM to promote fragmentation [49]. Specifically, these molecular events underlie host cell death induced by the vacuolating cytotoxin A (VacA) of *Helicobacter pylori* [50] as well as the secreted porin B (PorB) of *Neisseria gonorrhoea* [51-53]. Along similar lines, LLO from *L. monocytogenes* is responsible for mitochondrial fragmentation and the concomitant bioenergetics crisis that characterize the early phase of infection [54]. However, this occurs through a non-canonical DRP1-independent mechanism [55] that involves (at least in part) mitochondrial contact site and cristae organizing system subunit 10 (MICOS10) [42].

However, mitochondrial fission does not systematically correlate with apoptosis initiation, but rather participates in different pathophysiological processes, including metabolic adaptation [56] and the control of redox homeostasis [57]. For instance, a type 4 secretion system (T4SS) effector protein from *Legionella pneumophila* targets mitochondria and promotes rapid DRP1-dependent fission, but not overt RCD, initiating a series of mitochondrial alterations that ensure bacterial replication [58]. These might include reduced reactive oxygen species (ROS) generation [59] and chronic 5' AMP-activated protein kinase (AMPK) activation [60], which is also involved in the fragmentation-coupled mitophagy [61,62]. Moreover, deletion of mitofusin 2 (*MFN2*), which participates in mitochondrial fusion, from macrophages results in extensive mitochondrial fragmentation, altered respiration and reduced ROS levels, but no overt RCD, ultimately allowing for the survival and dissemination of both *L. monocytogenes* and *M. tuberculosis* [63]. A similar pathway of ROS inhibition has been documented in methicillin-resistant *S. aureus* (MRSA) [64]. In this latter case, however, the physical

dissociation between ROS-producing mitochondria and cytosolic pathogens appears to be involved in pathogenicity [64].

These observations delineate a mechanism whereby mitochondrial fission and subcellular re-localization limit ROS generation, either by facilitating the degradation of ROS-producing mitochondria by mitophagy [65], as observed for *Listeria spp.* [66], or by cooperating with other alterations including metabolic reprogramming (see above), ultimately setting up ideal conditions for bacterial growth. At least apparently at odds with this notion, *C. trachomatis* indirectly promotes mitochondrial elongation upon inhibiting fission via specific microRNAs (miRNAs) [67], ultimately boosting ATP production to sustain proliferation in epithelial cells [17]. Such a metabolic alteration is accompanied by an increase in ROS levels [68], but *C. trachomatis* exploits them for activating caspase 1 (CASP1) in further support of growth [69].

Specific cell types including macrophages promptly react to bacterial invasion by MFN1/MFN2-dependent fusion, a process that is required for optimal activation of defense mechanisms including autophagy and the secretion of antimicrobial cytokines [63,70,71]. Consistent with this notion, loss of DRP1 potentiates innate immune defenses driven by mitochondrial species such as ROS and mtDNA [72] (**Box 1**). Similar observations have also been made in adipocytes infected by the eukaryotic pathogen *Toxoplasma gondii*, although in this case the protective effects of mitochondrial fusion originate from the ability of elongated mitochondria to efficiently use fatty acids and hence restrict their availability for the pathogen [73]. Taken together, these observations suggest that most (but not all) bacteria harness mitochondrial fission as a strategy to control host mitochondria in support of proliferation and dissemination.

MAMs represent key hubs where mitochondrial dynamics are regulated [74], at least in part reflecting their versatility and multi-functional nature [75]. Suggesting that MAMs may also

be targeted by invading pathogens, Omp85 from *Neisseria spp.* controls lipid homeostasis in support of pathogenicity [76,77], and the human Omp85 human homolog SAMM50 sorting and assembly machinery component (SAMM50) actively participates in the regulation of MAM dynamics from the OMM [78,79]. Moreover, LegS2 (a T4SS effector from *L. pneumophila*) has been detected at both ER and mitochondrial membranes [80], indicating a putative MAM localization from which it might interfere with sphingolipid homeostasis and autophagy [81]. Yet another *L. pneumophila* effector (*i.e.*, Lpg1137) accumulates at MAMs to promote the degradation of the scaffold protein syntaxin 17 (STX17) and disrupt ER-mitochondria communication, ultimately inhibiting autophagy in support of pathogen survival [82]. Of note, *L. pneumophila* also secretes a factor commonly known as Legionella nucleotide carrier Protein (LncP) that integrates at the IMM to regulate ATP transport [83]. This event appears to contribute to pathogenicity at least in part by repressing autophagy [83]. In addition, *P. aeruginosa* actively inhibit autophagy in the context of cystic fibrosis, although in this case such an effect originates from the stabilization (rather than the destabilization) of ER-mitochondria contacts [84]. Whether this process involves quorum sensing (QS) molecules, which normally account for *Pseudomonas*-related mitochondrial dysfunctions [85,86], remains to be established.

Irrespective of these and other unsolved issues, mitochondrial dynamics and MAMs stand out as preferential targets for invading pathogens to survive and proliferate in host cells (**Fig. 3**), at least in part reflecting the central involvement of MAMs in autophagy regulation [87] and innate immune signaling (**Box 1**).

Mitophagy and cell death

Autophagy and RCD are critical for the preservation of intracellular and organismal homeostasis [3], respectively, hence representing major targets for host cell control by pathogenic bacteria. Active RCD induction by invading pathogens normally ensues replication, implying that it occurs in the context of failed pathogen control by autophagic responses [88]. As mitochondria are crucial players in specific variants of autophagy (mitophagy) and RCD (mitochondrial apoptosis and MPT-driven regulated necrosis), bacteria have evolved several strategies to control these processes at the mitochondrial level.

Bacterial invasion generally drives a pronounced autophagic response that targets not only the pathogen itself (so-called xenophagy) but also mitochondria damaged by infection (mitophagy) [25]. These responses rely on a common molecular machinery, but involve specific **autophagy receptors** [89]. For instance, the T3SS of *P. aeruginosa* promotes mitochondrial damage coupled to the release of ROS and mtDNA that is actively contained by mitophagy [90]. Pointing to an antimicrobial activity for these responses, *P. aeruginosa* developed multiple mechanisms to inhibit autophagy in host cells [91]. Conversely, *L. monocytogenes* actively drives mitophagy via LLO to quench ROS production in infected macrophages, which not only favors cytosolic pathogen persistence [66], but also inhibits phagocytosis [92]. Of note, LLO-driven mitophagy results from the direct interaction between LLO and the receptor NLR family member X1 (NLRX1) independent of the canonical pathway mediated by PTEN induced kinase 1 (PINK1), and hence preferentially occurs in cells with high NLRX1 levels such as macrophages [66].

For several bacteria, optimal pathogenicity involves the temporal control of RCD in host cells to enable pathogen replication in the context of inhibited cell death, but ultimately facilitates cellular breakdown in support of pathogen dissemination. Different effectors from the T4SS

of *L. pneumophila* initially prevent apoptotic RCD by neutralizing BAX and BAK1 [93-95], or inducing the ATP synthase to operate in reverse mode [96] to preserve mitochondrial transmembrane potential and avoid mitochondrial collapse. Conversely, at later stages of infection, *L. pneumophila* actively drive BAK1-independent cell death to facilitate bacterial dissemination [97]. pORF5 from *C. trachomatis* also inhibits RCD by attenuating mitochondrial dysfunction, as evidenced by reduced BAX levels, quenched release of cytochrome c, somatic (CYCS) into the cytosol and enhanced activation of the cytoprotective protein BCL2 apoptosis regulator (BCL2) [98]. Interestingly, this effect appears to originate from the upregulation of high mobility group box 1 (HGMB1), a conserved protein involved in autophagy and apoptosis regulation [98].

M. tuberculosis is well known for its capacity to promote the death of host cells in support of dissemination. One of the major mechanisms underlying such ability converges on Bcl-2 family proteins, especially the pro-apoptotic factor BCL2 like 11 (BCL211, best known as BIM), which can be activated by the pathogen ESX-1 secretion system and ESAT-6 protein [99]. Moreover, several mycobacterial proteins localize to the mitochondrial matrix and drive apoptosis in host macrophages by promoting ROS generation [100]. In the latter case, RCD may occur via MPT-driven necrosis rather than apoptosis, as the PTPC is particularly sensitive to ROS elevations [101]. Moreover, instances of mixed RCD scenarios involving both apoptotic and necrotic components have been documented in the context of *S. aureus* infection [102,103]. Other bacteria have been shown to employ a range of mechanisms to promote mitochondrial RCD. These include *S. flexneri*, which drives RCD in host cells via invasion plasmid antigen D (IpaD) [104], as well as the extracellular bacteria *Acinetobacter baumannii* and the nosocomial pathogen *Stenotrophomonas maltophilia*, whose cytotoxicity involves the outer membrane protein A (OmpA) [20,105,106]. Similarly, outer membrane vesicles (OMVs) from *N. gonorrhoeae*, uropathogenic *Escherichia coli* and *P. aeruginosa*

have all been shown to drive mitochondrial RCD in macrophages upon depletion of MCL1 apoptosis regulator, BCL2 family member (MCL1) [53].

Taken together, these observations exemplify various strategies through which bacterial pathogens control mitophagy and RCD at the level of mitochondria (**Fig. 4**).

Concluding remarks

Most, if not all, pathogenic bacteria have evolved a panel of strategies to control mitochondrial functions in host cells, largely reflecting the key position that mitochondria occupy in virtually all facets of the biology of modern eukaryotes [24]. It is tempting to speculate that the predisposition of bacteria to converge prevalently on mitochondria rather than other organelles, such as the ER or lysosomes, reflects the evolutionary origin of mitochondria. Indeed, mitochondria emerged from an endosymbiotic interaction between ancestral alpha proteobacteria (the evolutionary ancestors of modern Gram-negative bacteria) [107]. This implies that (at least some degree of) structural or functional homology between modern mitochondria and contemporary bacteria persisting throughout evolution may explain (at least some of the) interactions between mitochondrial component and pathogen factors. However, such a possibility does not directly account for the multipronged interactions between modern Gram-positive bacteria and eukaryotic pathogens with mitochondria. Thus, modern pathogens may simply have evolved strategies to control host mitochondria as a direct consequence of the robust evolutionary pressure existing on key processes that impinge on mitochondrial activity, including (but perhaps not limited to) RCD and innate immune signaling. Although other issues still remain open (*see* **Outstanding Questions**), the functional interactions between invading bacteria and host mitochondria stand out as key aspects of pathogenicity. Additional studies are needed to elucidate the therapeutic potential of drugs specifically conceived to disrupt such bacteria-mitochondria intersections.

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Box 1. Innate immune signaling by mitochondria.

The ability of cells to engage the immune system in response to stressful conditions, including (but not limited to) pathogenic challenges, is intimately associated with mitochondrial functions, thereby constituting a key link between intracellular and organismal homeostasis. For instance, mitochondrial derangements caused by infection can lead to the buildup of reactive oxygen species (ROS) and mitochondrial DNA (mtDNA) molecules in the cytoplasm, culminating with the activation of the NLR family pyrin domain containing 3 (NLRP3), NLR family CARD domain containing 4 (NLRC4) or absent in melanoma 2 (AIM2) inflammasome and consequent secretion of mature interleukin 1 beta (IL1B, best known as IL-1β) and IL-18. Alternatively, oxidized mtDNA accumulating in the cytosol of infected cells can promote cyclic GMP-AMP synthase (CGAS) and stimulator of interferon response cGAMP interactor 1 (STING1) signaling, resulting in the initiation of NF-κB- and interferon regulatory factor 3 (IRF3)-dependent pro-inflammatory transcriptional programs. Moreover, the mitochondrial antiviral signaling protein (MAVS), a key signal transducer in various pathways that detect cytosolic double-stranded RNA to initiate antiviral responses based on type I interferon (IFN), is localized to the outer mitochondrial membrane (OMM). Finally, several mitochondrial components including mtDNA, transcription factor A, mitochondrial (TFAM) and the inner mitochondrial membrane (IMM)-restricted lipid cardiolipin mediate robust immunostimulatory signals once released in the extracellular environment, *de facto* operating as damage-associated molecular patterns (DAMPs) even in the absence of infectious challenges [4,108].

Glossary

Mitochondrial permeability transition (MPT). Rapid alteration in the permeability of the inner mitochondrial membrane that causes a specific variant of regulated necrosis.

Autophagy. Evolutionary conserved process ensuring the removal of dispensable or cytotoxic cytosolic material through lysosomal degradation.

Mitophagy. A variant of autophagy that selectively targets permeabilized or otherwise dysfunctional mitochondria.

Tricarboxylic acid (TCA) cycle. A series of chemical reactions that converts acetyl-CoA into NADH and $FADH_2$ in support of oxidative phosphorylation and ATP production by mitochondria.

Permeability transition pore complex (PTPC). The multicomponent pore of the inner mitochondrial membrane responsible for mitochondrial permeability transition (MPT).

Mitochondria-associated ER membranes (MAMs). Specialized regions of the endoplasmic reticulum membrane that are physically and functionally connected to mitochondria.

LC3-associated phagocytosis (LAP). LC3-dependent process that enables the uptake and lysosomal degradation of extracellular pathogens and dead cell corpses.

Fission. Multistep process through which mitochondria split in support of replication or mitophagy.

Fusion. Multistep process through which mitochondria fuse in support of improved bioenergetic metabolism or mtDNA redistribution.

Autophagy receptors. Components of the autophagy machinery involved in the recognition of specific substrates destined to lysosomal degradation.

Table 1. Control of mitochondrial functions by pathogens.

Pathogen	Bacterial factor(s)	Mitochondrial target(s)	Cellular response	Outcome	Ref.
Acinetobacter baumannii	OmpA	DRP1	Mitochondrial fragmentation ROS production	Host cell death induction	[20]
Brucella abortus	-	-	Metabolic rewiring	Improved pathogen survival	[13]
Chlamydia trachomatis	pORF5	BAX BCL2	Limited CYCS release Apoptosis inhibition	Improved pathogen survival	[98]
	-	-	Metabolic rewiring	Improved pathogen survival	[18]
Helicobacter pylori	VacA	DRP1	Mitochondrial fission BAX activation	Host cell death induction	[50]
	VacA	-	Reduced BCL2 expression	Host cell death induction	[109]
Legionella pneumophila	Lpg1137	STX17	Autophagy inhibition	Improved pathogen survival	[82]
	SidF	BNIP3	BNIP3 inhibition	Improved pathogen survival	[93]
	MitF	DRP1	Mitochondrial fission Metabolic rewiring	Improved pathogen survival	[58]
	LncP	-	ATP transport	Improved pathogen survival	[83]
	VipD	-	$\Delta\psi$ loss CYCS release	Host cell death induction	[97]
Listeria monocytogenes	LLO	NLRX1	Inhibition of ROS production Mitophagy inhibition	Improved pathogen survival	[66]
	LLO	MICOS10	Mitochondrial fission	Improved pathogen survival	[42,54]
Mycobacterium tuberculosis	ESX-1 Rv01654	BIM	Caspase activation	Host cell death induction	[99,110]
	Rv0674	mtDNA	ROS production Inhibition of ATP production	Improved pathogen survival	[100]
	Rv0694 ESAT-6	-	Metabolic rewiring	Improved pathogen survival	[14,16]
Neisseria gonorrhoeae	PorB	-	Ultrastructural defects	Host cell death induction	[51]
	PorB	-	$\Delta\psi$ loss CYCS release	Host cell death induction	[52]
	OMV	-	MOMP	Host cell death induction	[53]
Pseudomonas aeruginosa	T3SS	NLRC4 inflammasome	mtDNA release ROS production	Improved pathogen survival	[90]
	ExoS	-	Autophagy inhibition	Improved pathogen survival	[91]
	OMV	-	MOMP	Host cell death induction	[53]
Shigella flexneri	IpaD	-	Caspase activation	Host cell death induction	[104]
	-	BCL2	ROS production $\Delta\psi$ loss	Host cell death induction	[31]
Staphylococcus aureus	PVL	-	MPT Caspase activation	Host cell death induction	[103]
	Alpha-toxin	NRLP3 inflammasome	Separation of mitochondria from intracellular bacteria	Improved pathogen survival	[64]
	-	-	Metabolic rewiring	Improved pathogen survival	[21]
Stenotrophomonas maltophilia	OmpA	BAX	ROS production Intracellular Ca^{2+} increase	Host cell death induction	[106]

Abbreviations: $\Delta\psi$, mitochondrial transmembrane potential; MOMP, mitochondrial outer membrane permeabilization; MPT, mitochondrial permeability transition; mtDNA, mitochondrial DNA; OMV, outer membrane vesicles; ROS, reactive oxygen species; T3SS, type 3 secretion system.

Legends to Figures

Figure 1. **Bacterial control of mitochondrial metabolism in host cells.** *Brucella abortus* favors glucose uptake via plasma membrane glucose transporters (GLUTs) as it inhibits oxidative phosphorylation (OXPHOS), culminating with lactate accumulation in support of pathogen viability (**A**). *Pseudomonas aeruginosa* harnesses succinate accumulating as a result of impaired OXPHOS in cells with CF transmembrane conductance regulator (*CFTR*) mutations in support of colonization (**B**). *Mycobacterium tuberculosis* has been proposed to inhibit both glycolysis and OXPHOS and rely on the uptake of exogenous fatty acids for survival (black) as well as to divert glycolysis towards the production of ketone bodies (KBs) in support of fatty acid accumulation (red) (C). *Staphylococcus aureus* and *Chlamydia trachomatis* boost both glycolysis and OXPHOS to exacerbated intracellular ATP availability.

Figure 2. **Alterations of mitochondrial Ca^{2+} homeostasis imposed by invading pathogens.** *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Shigella flexneri* can all mobilize Ca^{2+} ions from the endoplasmic reticulum (ER) via inositol 1,4,5 trisphosphate receptor (IP_3R) channel, resulting in the accumulation of Ca^{2+} in the mitochondrial matrix via voltage-dependent anion channel (VDACs) at the outer mitochondrial membrane (OMM) and the mitochondrial calcium uniporter (MCU) complex at the inner mitochondrial membrane (IMM). However, while *S. aureus* and *S. pneumoniae* do so in an accentuated manner, rapidly resulting in innate immune signaling via cytosolic mitochondrial DNA (mtDNA) regulated cell death (RCD), the type 3 secretion system (T3SS) effector IpgD from S. *flexneri* actively prevents RCD in the early phases of infection upon binding to IP_3R channels (**A**). During *Listeria monocytogenes* infection, mitochondrial Ca^{2+} waves promote the activation of the pyruvate dehydrogenase (PDH) complex, ultimately resulting in accumulation of acetyl-CoA (Ac-CoA), acetylation of rubicon (RUBCN) and inhibition of LC3-associated phagocytosis

(LAP) (**B**). BAK1, BCL2 antagonist/killer 1; BAX, BCL2 associated X, apoptosis regulator; CYCS, cytochrome c, somatic; MAMs, mitochondria-associate ER membranes; MICU1, mitochondrial calcium uptake 1, LLO, listeriolisin O.

Figure 3. **Shifts in mitochondrial dynamics upon bacterial infections.** Numerous pathogenic bacteria encode factors that promote mitochondrial fission, via mechanisms that may or may not involve dynamin 1 like (DNM1L, best known as DRP1). This can support metabolic rewiring in host cells, favor mitophagy or precipitate cell death (**A**). Conversely, *Chlamydia trachomatis* promotes mitochondrial elongation (by inhibiting fission) in support of increased ATP production via a hitherto unidentified factor (**B**). LLO, listeriolisin O; PorB, porin B; VacA, vacuolating cytotoxin A.

Figure 4. **Control of mitophagy and RCD by invading pathogens.** Mitochondrial damage caused by *Pseudomonas aeruginosa* and *Listeria monocytogenes* is associated with an increase in reactive oxygen species (ROS) that drives NLR family member X1 (NLRX1)-dependent mitophagy (**A**). A variety of bacterial species encoded cytotoxic and cytoprotective factors that interact with the molecular machinery causing mitochondrial outer membrane permeabilization (MOMP) or mitochondrial permeability transition (MPT) to control caspase-dependent and caspase-independent forms of regulated cell death (**B**). $\Delta\psi_m$, mitochondrial transmembrane potential; BAK1, BCL2 antagonist/killer 1; BAX, BCL2 associated X, apoptosis regulator; CYCS, cytochrome c, somatic; PTPC, permeability transition pore complex.

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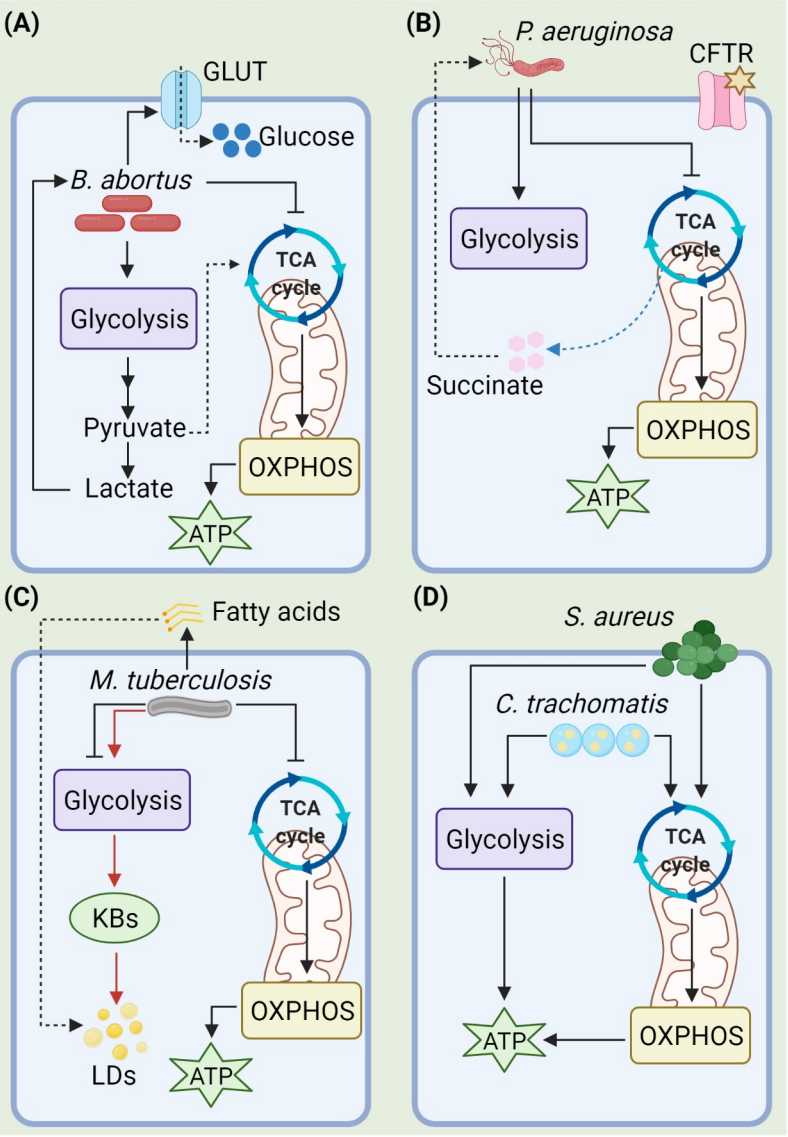
(A)
GLUT
Glucose
B. abortus
Glycolysis
TCA cycle
Pyruvate
Lactate
OXPHOS
ATP
(B)
P. aeruginosa
CFTR
Glycolysis
TCA cycle
Succinate
OXPHOS
ATP
(C)
Fatty acids
M. tuberculosis
Glycolysis
TCA cycle
KBs
LDs
OXPHOS
ATP
(D)
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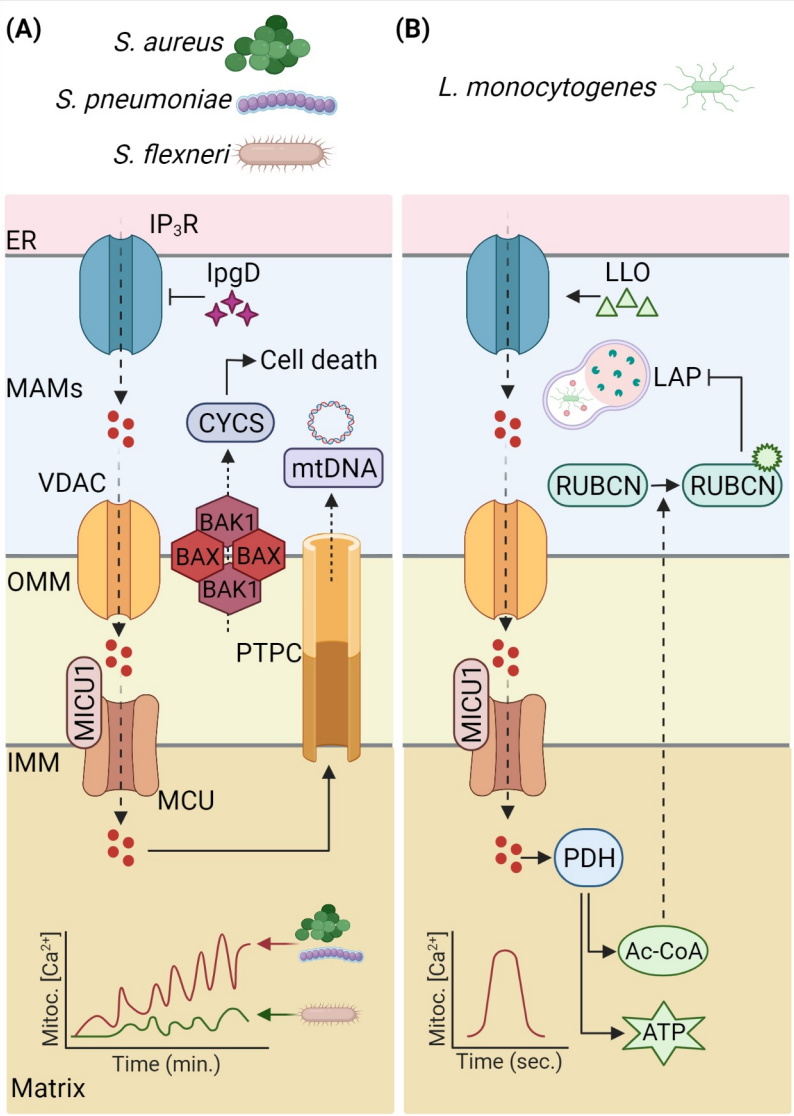
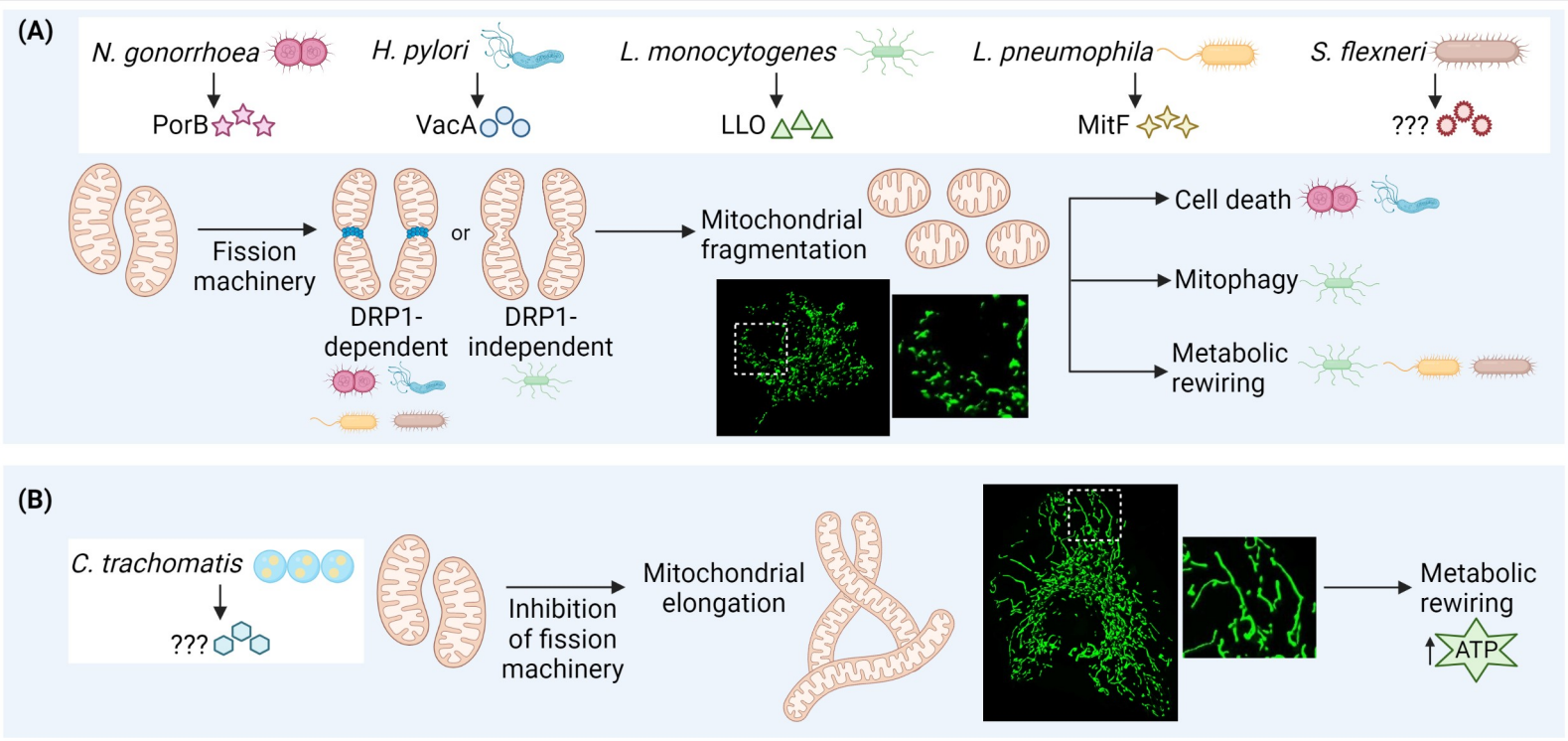
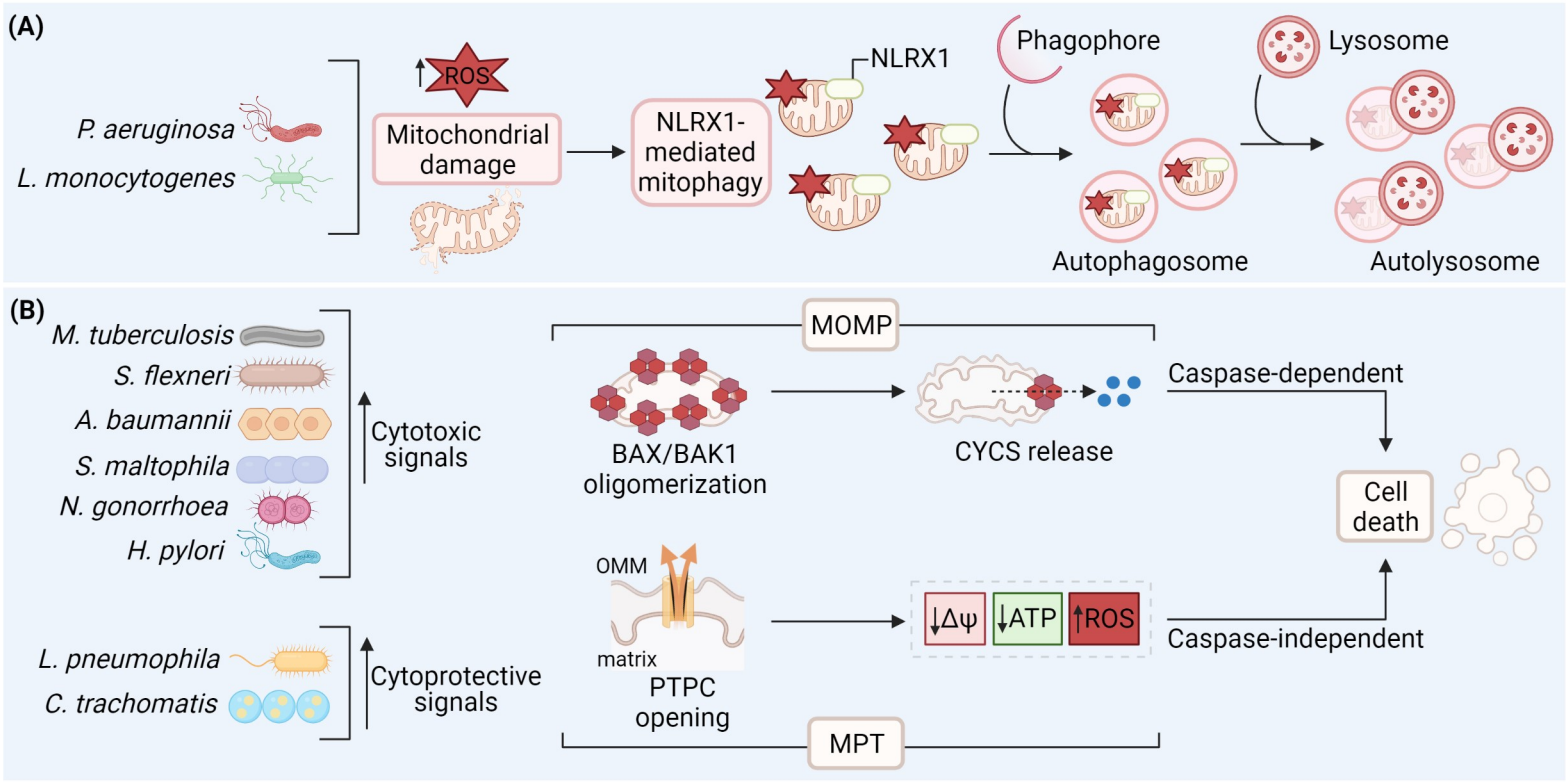
C. trachomatis
Glycolysis
TCA cycle
ATP
OXPHOS


(A)
S. aureus
S. pneumoniae
S. flexneri
(B)
L. monocytogenes
ER
IP3R
IpgD
Cell death
MAMs
CYCS
mtDNA
VDAC
BAK1
BAX
BAX
BAK1
OMM
PTPC
MICU1
IMM
MCU
Mitoc. [Ca2+]
Time (min.)
Matrix
LLO
LAP
RUBCN
RUBCN
MICU1
PDH
Ac-CoA
ATP
Mitoc. [Ca2+]
Time (sec.)


(A)
N. gonorrhoea
PorB
H. pylori
VacA
L. monocytogenes
LLO
L. pneumophila
MitF
S. flexneri
???
Fission machinery
DRP1-dependent
or
DRP1-independent
Mitochondrial fragmentation
Cell death
Mitophagy
Metabolic rewiring

(B)
C. trachomatis
???
Inhibition of fission machinery
Mitochondrial elongation
Metabolic rewiring
ATP


(A)
P. aeruginosa
L. monocytogenes
ROS
Mitochondrial damage
NLRX1-mediated mitophagy
NLRX1
Phagophore
Lysosome
Autophagosome
Autolysosome
(B)
M. tuberculosis
S. flexneri
A. baumannii
S. maltophila
N. gonorrhoea
H. pylori
Cytotoxic signals
L. pneumophila
C. trachomatis
Cytoprotective signals
MOMP
BAX/BAK1 oligomerization
CYCS release
Caspase-dependent
Cell death
OMM
matrix
PTPC opening
↓Δψ
↓ATP
↑ROS
Caspase-independent
MPT