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Organelle targeting during bacterial infection: Insights from *Listeria*

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Abstract

Listeria monocytogenes, a facultative intracellular bacterium responsible for severe foodborne infections, is now recognized as a multifaceted model in infection biology. Comprehensive studies of the molecular and cellular basis of the infection have unravelled how the bacterium crosses the intestinal and feto-placental barriers, invades several cell types in which it multiplies and moves, and spreads from cell to cell. Interestingly, although *Listeria* does not actively invade host cell organelles, it can interfere with their function. We discuss the effect of *Listeria* on the ER and the mechanisms leading to the fragmentation of the mitochondrial network and its consequences, and review the strategies used by *Listeria* to subvert nuclear functions, more precisely to control host gene expression at the chromatin level.

Highlights

- Organelle manipulation by *Listeria* determines the outcome of infection.
- Disruption of mitochondrial dynamics affects the efficiency of *Listeria* infection.
- Bacteria secrete nucleomodulins to reprogram host cell transcription.
- *Listeria*-induced perturbations in ion homeostasis impact all organelles.

Bacterial life in the complex intracellular environment

Intracellular bacteria have developed various strategies to invade, survive, and multiply inside cells. Many bacterial pathogens including *Legionella*, *Chlamydia*, *Salmonella* and *Brucella* co-opt inner membranes to create a specific compartment adapted to their needs [reviewed in 1,2]. Few bacteria, most of which are endosymbionts, reside inside pre-existing organelles [e.g. 3-5]. By contrast, several pathogenic species such as *Listeria monocytogenes*, *Shigella flexneri* and members of the genus *Rickettsia*, escape from their internalization vacuoles and complete their life cycle in the cytosol [reviewed in 6]. The respective benefits and detriments of these diverse habitats concerning nutrient availability or sensitivity to host immune recognition are difficult to compare. In each case, bacteria have evolved survival strategies adapted to their specific lifestyles. Despite of their different residence sites, most intracellular bacteria directly or indirectly interact with organelles and perturb their function. We illustrate this issue with the example of *Listeria monocytogenes*.

Organelles are among the most prominent features that distinguish eukaryotic from prokaryotic cells (see Glossary). They form intracellular compartments delimited by at least one membrane. They are defined by a characteristic composition and specific metabolic pathways. Organelles can furthermore act as platforms for signalling pathways, allowing the coordination of activities such as metabolism, innate immune sensing, and the stress response [7,8]. Therefore, they represent attractive targets for bacterial pathogens to hijack host cell function and ensure their own survival.

L. monocytogenes (hereafter also *Listeria*) is a food-borne bacterial pathogen capable of invasion and replication in phagocytic and non-phagocytic cells. This capacity allows it to cross the protective epithelial barriers of the human body and cause severe disease in immunocompromised individuals [reviewed in 9,10]. To invade host cells, *Listeria* expresses several dedicated proteins, notably internalin (InlA) and InlB. When the bacterium contacts the host cell membrane, InlA and InlB bind to E-Cadherin and to the hepatocyte growth factor (HGF) receptor Met respectively and induce bacterial internalization [reviewed in 11]. An internalization vacuole is formed from which the bacterium usually escapes; however, *in vivo* analysis of the crossing of the intestinal barrier has shown that *Listeria* can also transcytose across epithelial cells [12].

To evade membrane-bound structures, *Listeria* secretes two phospholipases (PlcA and PlcB), as well as a cholesterol-dependent cytolysin, listeriolysin O (LLO) [13-15]. When the pH of the internalization vacuole acidifies, LLO forms pores in the vacuolar membrane and destabilizes it. In addition to its role in vacuole escape, LLO acts at several steps of the infection cycle and appears as a multifunctional toxin [see 16 for a recent review].

An alternative pathway implicating the intravacuolar replication of *Listeria* has been documented in macrophages of mice with severe combined immunodeficiency [17]. The formation of non-acidic, non-degradative compartments named spacious *Listeria*-containing phagosomes (SLAPS) is dependent on macroautophagy, and on moderate secretion of LLO. SLAPs have been proposed to provide a niche for persistent *Listeria* infection within macrophages, but do not represent a general rule for intracellular proliferation.

Listeria does not only escape the internalisation vacuole, but also avoids autophagic recognition, entrapment in autophagosomes and degradation. During cellular infection, the membrane damage induced by LLO leads to a transient amino-acid starvation, to which the host cell responds through inhibition of the mTOR pathway and induction of autophagy [18,19]. However, PlcA and PlcB inhibit the cell autophagic flux; by stalling pre-

autophagosome structures, they protect bacteria from clearance [19]. Another player in escape from autophagy is the *Listeria* surface protein ActA, which recruits the actin polymerisation machinery onto the bacterial surface to promote intracellular movement and spreading to neighbouring cells [20,21]. The recruitment of actin by ActA [22] and that of the Major Vault Protein (MVP) by InlK [23] cover the bacterial surface and prevent the sensing and targeting of bacteria to autophagy [reviewed in 24].

Apart from its ability to escape from internalisation compartments and autophagosomes, *Listeria* has developed a broad range of strategies allowing it to interfere with the function of *bona fide* cellular organelles, *i.e.* pre-existing the bacterial infection. These activities range from the perturbation of vesicle trafficking, or of the ER, to more elaborate mechanisms leading to fragmentation of the mitochondrial network, or to the modulation of host gene expression via direct chromatin-targeting mechanisms. We review here our current understanding of these processes and of the benefit they convey to the bacterium.

Perturbation of the endomembrane system during *Listeria* infection

The endomembrane system defines the subset of cellular compartments enclosed by membrane structures that are involved in transport. It includes the ER, the Golgi apparatus, lysosomes, endo- and exocytic compartments, as well as the nuclear envelope. The different components of these systems can exchange membrane and contents through vesicular transport. During infection, *Listeria* interacts successively with several of these compartments.

Inhibition of phagosomal killing

A primary strategy of the cell to counteract invading bacteria is maturation and then fusion of the phagocytic vacuole containing the bacteria (the phagosome) with lysosomes, a pathway that is particularly active in macrophages (Fig. 1). The small GTPase Rab5a is associated with the membrane of early endosomes and phagosomes and recruits effectors required for phagosome maturation, including enzymes leading to phosphoinositide conversion [25,26]. Rab5a has also been reported to play a role in the killing of *Listeria* after uptake by macrophages, by inducing the translocation of the small Rho GTPase Rac to the phagosome, which in turn promotes the assembly, at the phago-lysosomal membrane, of a NADPH oxidase complex producing reactive oxygen species (ROS) in the mature phagosome [27]. By targeting Rab5a, *Listeria* blocks the maturation of phagosomes and thus avoids killing. Towards this aim, *Listeria* secretes a 40-kDa protein annotated as a glyceraldehyde-3-phosphate dehydrogenase (Lmo2459). Lmo2459 can mediate the ADP-ribosylation of Rab5a, which impedes phago-lysosomal maturation [28]; however, how Lmo2459 reaches its target Rab5a has not been defined. Whether a similar scenario occurs in epithelial cells is also unknown.

Inhibition of lysosome fusion

In addition to the mechanism described above, the pore-forming activity of LLO contributes to impede the fusion of lysosomes with the endocytic vacuole [29,30] (Fig. 1). Before bacterial escape into the cytosol, LLO-mediated perforation of the phagosomal membrane allows ion fluxes between compartments, with a notable drop in calcium concentration and an increase in pH inside the phagosome. This perturbation of the ion gradient across the phagosomal membrane inhibits fusion with LAMP1-positive lysosomes,

as previously reported for the depletion of vacuolar calcium [31]. One should note that the activity of LLO itself is sensitive, in addition to pH variations [32], to other vacuolar ion concentrations, such as K^+/Cl^- [33]. In line with this, the host chloride channel Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) located in the phagosomal membrane promotes *Listeria* phagosomal escape by increasing intraphagosomal Cl^- concentration and thereby potentiating LLO oligomerization [33].

Altogether, by blocking the maturation of endocytic compartments, LLO and Lmo2459 allow the bacteria to avoid intraphagolysosomal degradation.

Perturbation of intracellular calcium storage and signalling

LLO triggers a massive increase in cytosolic calcium concentration, primarily due to an influx of extracellular Ca^{2+} via pores in the plasma membrane [34]. Ca^{2+} influx has been shown to promote bacterial entry, possibly through the fusion of subcortical vesicles that increase the membrane availability required for the uptake of a particle as large as a bacterium [35,36]. In addition, compartments of the endomembrane system, such as the ER, release stored Ca^{2+} into the cytosol upon infection. This release relies on two LLO-dependent mechanisms. First, InlB and LLO trigger signalling cascades including protein tyrosine kinases, which result in the activation of phospholipases C (PLC) and the generation of inositol triphosphate (IP_3), thereby stimulating the IP_3 receptor Ca^{2+} channels [37,38]. Second, LLO is responsible for reversible damages to the ER and lysosome membranes and the release of their content [30,38], inducing a transient increase in cytosolic calcium concentration. In both epithelial and immune cells, LLO-induced Ca^{2+} signals were shown to lead to a production of pro-inflammatory cytokines, which may contribute to pathology in different tissues, such as the intestine and the liver [38-40].

Activation of the unfolded protein response/ER stress

The ER damage mediated by LLO even prior to bacterial entry causes rapid swelling and loss of ER-tracker staining, which is typical of ER stress [38]. This stress is likely amplified by the concomitant increase in cytosolic calcium concentration [38,41]. In response to this stress, the unfolded protein response (UPR) is activated, and ultimately leads to cell death if the infection is sustained [30,38,42]. This cell response is considered to be antibacterial, as induction of the UPR prior to infection reduces the bacterial number [42].

Similar to the ER-based UPR, the mitochondrial UPR (mtUPR) is induced upon mitochondrial stress, where the protein ATFS1 accumulates in the nucleus instead of mitochondria and activates the transcription of proteins exerting protective functions in mitochondria [38,43]. The mtUPR pathway was recently found to activate an innate immunity program, suggesting that surveillance of organelle homeostasis is part of the active antimicrobial strategy in infected cells [44]. It remains to be uncovered whether this pathway is activated upon *Listeria* infection.

Mitochondrial dynamics and infection

Mitochondria are essential organelles that form a dynamic network within the cell. They play a crucial role in energy metabolism and cellular signalling pathways such as innate immunity, calcium signalling or apoptosis, which makes them an attractive target for pathogens to interfere with cellular function. Indeed, several bacteria and viruses modulate mitochondrial function to maintain their replicative niche by preventing host cell apoptosis or

to favour dissemination by inducing apoptosis [reviewed in 45-47]. A variety of physiological and pathological conditions have been shown to induce either fusion or fission of the mitochondrial network and thereby affect mitochondrial function.

Infection with *L. monocytogenes* induces the host cell mitochondrial network to undergo a rapid, transient fragmentation already at early time points of infection (1-3h), along with a loss in the mitochondrial membrane potential, which results in decreased intracellular ATP levels [48] (Fig. 2A). This effect is specific to pathogenic *Listeria*, as it is not observed with the non-pathogenic species *L. innocua* or several other intracellular bacteria. The main bacterial factor responsible for mitochondrial fragmentation is the pore-forming toxin LLO, which causes calcium influx across the plasma membrane, although the exact signalling pathway leading to mitochondrial fragmentation is yet to be discovered. Other pore-forming toxins from the same family as LLO, such as pneumolysin, perfringolysin or streptolysin induce the same morphological phenotype as LLO, but it is largely unknown whether and how infection with the corresponding bacteria would affect mitochondria. In contrast to the bacterial pore-forming proteins *Helicobacter pylori* VacA, *Neisseria gonorrhoeae* PorB or *Staphylococcus aureus* Panton-Valentine leukocidin [reviewed in 46] (Fig 2B), which reach and perturb mitochondrial membranes, LLO does not appear to localize to mitochondria, although it might do so if delivered by bacterial membrane vesicles, as has been recently shown for the enteropathogenic *Escherichia coli* Hly toxin [49].

Strikingly, functional mitochondrial dynamics appears to be important for *Listeria* infection, because perturbing the fusion-fission balance by siRNA against key components of the fusion machinery causes a decrease in the infection efficiency, while interfering with fission results in its augmentation. Together, these findings led to the hypothesis that *Listeria* targets the host cell mitochondrial network to temporarily shut down cellular bioenergetics and to interfere with mitochondrial innate immune signalling [48]. Current efforts are aimed at obtaining a global view of the mitochondrial pathways that are reorganized upon *Listeria* infection (Box 1).

Mechanistically, the atypical mitochondrial fragmentation induced by *Listeria* differs from VacA-induced mitochondrial fragmentation [50], since it is independent of the key mitochondrial fission protein, the dynamin-related protein Drp1, which dissociates from mitochondria upon LLO treatment [51]. As in canonical fragmentation, the ER and actin were found to contribute significantly to the process; however, the fragmentation sites were marked by the ER also in the absence of Drp1, suggesting that although the initial constriction machinery is shared, molecules other than Drp1 can terminate mitochondrial abscission downstream of the ER. Uncovering such molecule(s) is a challenging issue, since to date LLO and *Listeria* are the only known exogenous stimuli of Drp1-independent mitochondrial fragmentation (Box 1).

Recently, mitochondria have been shown to play an important role in innate immunity through Mitochondrial AntiViral Signalling protein (MAVS), an adaptor protein located on the mitochondrial outer membrane that regulates signal transduction from cytosolic RNA sensors. MAVS activity has been shown to depend on mitochondrial dynamics and function [52-54]. Through the assembly of a large signalling complex on mitochondria, activated MAVS triggers nuclear translocation of the transcription factors NF- κ B, IRF3 and IRF7, which results in the production of interferons, cytokines and interferon-stimulated genes (ISGs) [reviewed in 55]. Similar to other mitochondrial tail-anchored proteins, MAVS has additionally been found to localize at peroxisomes [56]. MAVS is one of the mediators of the innate immune response to *Listeria* in epithelial cells, leading to the production of type I and type III interferons [57,58]. Consistent with the disruption of mitochondrial morphology and

function by *Listeria*, it appears that MAVS-dependent stimulation of the interferon response is mainly carried out by peroxisomal rather than mitochondrial MAVS [58].

Targeting nuclear functions: strategies to reprogram the host

Upon infection by *Listeria*, the transcriptome of host cells is vastly reshaped. Different patterns of transcriptional response have been identified, depending on the nature of target cells in experiments performed *in vitro* [59-62], or on more complex regulatory circuits occurring in mouse models [63,64]. This reprogramming is largely the consequence of cellular signalling events occurring upon bacterial entry and/or subsequent recognition of Pathogen Associated Molecular Patterns (PAMPs) by a variety of host sensors named Pattern-Recognition Receptors (PRRs) [reviewed in 65]. In addition to these pathways, the notion recently emerged that pathogenic bacteria could manipulate host transcription by directly acting at the nuclear level and interfering with chromatin-based regulation [66,67], giving rise to the emerging field of patho-epigenetics (Box 1). Research on *Listeria* has been on the leading edge of this topic. As documented below, it has revealed that bacterial-triggered signalling events can impact the post-translational modification of histone tails [68], and that a virulence factor, belonging to a new class of bacterial secreted effectors named nucleomodulins [69] can localize to the cell nucleus and perturb chromatin silencing.

Perturbation of histone modifications

Eukaryotic DNA is organised in the nucleus into a structure named chromatin [reviewed in 70]. The primary unit of chromatin architecture is the nucleosome, which consists of a core octamer of histones H2A/B, H3 and H4, around which DNA wraps together with linker histones H1. Successive nucleosomes are packed more or less tightly to form chromatin fibres. The post-translational status of core histone tails plays a key function in the organization of this structure; by modulating the compaction of the chromatin assembly, and acting as specific docking sites for regulatory factors, these modifications constitute a histone code that adds another layer in the regulation of gene expression and cell fate decisions [71].

LLO induces histone deacetylation and dephosphorylation

Listeria was one of the first examples of an invasive bacterium reported to have an effect on its host's histone modifications [72]. Following the infection of HeLa cells, the total levels of serine 10 phosphorylation histone H3 (H3S10deP) and lysine acetylation of histone H3 (H3S10deP), as well as acetylation of histone H4 (H4KdeAc) are significantly decreased (Fig. 3, *left*). Both H3S10deP and H4KdeAc are induced by secreted LLO, and can be recapitulated by addition of the purified toxin. Transcriptional studies have highlighted that a specific subset of host genes is de-regulated within 20 minutes of LLO treatment. Interestingly, Chromatin Immunoprecipitation (ChIP) experiments have revealed that H3S10deP and H4KdeAc occur specifically at promoter genes that are differentially regulated upon LLO addition, suggesting that histone modifications induced by LLO are responsible for the reprogramming of host gene expression. In terms of mechanism, the intracellular signalling leading to H3S10deP is dependent on potassium efflux driven by increased membrane permeability upon LLO treatment, and can be recapitulated with other bacterial pore-forming toxins or potassium-specific ionophores [73]. Future investigations are needed to clarify how the decrease in cellular potassium concentration is sensed, and which signalling pathways result in histone modifications.

Histone deacetylation by SIRT2

In contrast to H3S10deP and H4KdeAc, H3KdeAc has been shown to be a LLO-independent process [72]. It is driven by another virulence factor, InlB, one of the two surface internalins involved in *Listeria* entry into its target cells [74]. Upon binding to its cell membrane receptor Met (the receptor of HGF), InlB triggers the activation of a signalling cascade *via* the PI3K/Akt pathway, which results in the translocation of the tubulin-deacetylase SIRT2 [75] from the cytoplasm to the chromatin fraction of the nucleus (Fig. 3, *right*). There, SIRT2 is responsible for the specific deacetylation of lysine 18 of H3 during infection, and for the transcriptional deregulation of hundreds of host genes. ChIP experiments showed that the promoter of genes that are repressed in a SIRT2-dependent manner during infection are enriched in deacetylated H3K18, suggesting that the activity of the histone deacetylase at these promoters is responsible for their repression during infection. The subversion of SIRT2 function by InlB proves to be pro-bacterial, as SIRT2^{-/-} mice can control listeriosis more efficiently than their wild type littermates. Further studies will be required to identify which SIRT2-regulated genes are most important for host defence against *Listeria*. Given that *Listeria* is the only known stimulus that induces SIRT2 translocation to the nucleus, it will provide an essential tool to elucidate the precise mechanisms mediating SIRT2 regulation at the chromatin.

Interaction of the nucleomodulin LntA with the heterochromatin protein BAHD1

Upon infection of epithelial cells, *Listeria*, like other intracellular bacteria, activates host innate immune responses, which lead to the production and secretion of a variety of cytokines including type I and III interferons [reviewed in 76]. However, the expression of downstream ISGs is repressed by the heterochromatinization factor, BAHD1 [77] (Fig. 3, *bottom*). In non-infected cells, BAHD1 can nucleate the formation of heterochromatin at the promoter of a subset of host genes [78]; during infection, BAHD1 additionally targets ISGs – via a so-far unknown mechanism. *Listeria* can, under conditions that are still poorly defined, secrete the nucleomodulin LntA into the cell cytosol. This protein enters the host nucleus where it binds directly to BAHD1, causing it to dissociate from chromatin, and thus activating the expression of ISGs [77,79]. The *lntA* virulence gene, and a tight regulation of its expression, is required for an optimal infectious process *in vivo*, indicating that the deregulation of BAHD1 must be carefully timed and tuned. In line with this hypothesis, hypomorphic BAHD1^{+/-} mice are more resistant to *Listeria* infection than their wild type littermates. The precise effects of the host interferon responses on the patho-physiological outcomes of infection is still a matter of debate [80]; their study will certainly lead to surprising findings, which should clarify why their accurate modulation is essential to the host as well as to the bacterium.

Other secreted effectors of *Listeria* that can translocate to the host cell nucleus are currently under investigation. Their extensive functional characterisation should highlight novel mechanisms of subversion of nuclear functions by *Listeria* nucleomodulins.

Concluding remarks

The strategies of organelle subversion by *Listeria* that we have reviewed here reveal some of the ingenious tricks allowing the bacterium to adapt to its intracellular environment. Via a variety of molecular mechanisms, the bacterium is able to reorganize the host cell endomembrane dynamics, its energetics and gene expression program, most often for its own

profit. Depending on the organelle, strategies and consequences differ significantly. For instance, mitochondrial targeting results in a global reshaping of their structure, dynamics and functionality, and we are only beginning to understand the functional consequences of these events; they might represent the mammalian counterpart to the surveillance pathway described in *Caenorhabditis elegans*, which correlates mitochondrial dysfunction with pathogen attack [81].

In contrast to the profound effect on mitochondria, the harnessing of chromatin by the *Listeria* protein LntA appears as a more focused process. It allows *Listeria* to selectively control the transcription of a subset of host genes, like ISGs, independently from other nuclear functions affected by infection, such as the DNA damage response described in other studies [82,83].

Organelle targeting during infection is not restricted to *L. monocytogenes*; a number of viruses, parasites and intracellular bacteria seem to have evolved alternative approaches to hijack the functions of metazoan organelles such as mitochondria or nuclei [see for instance 84-87], of which nuclear targeting has been previously reviewed [69]. As exemplified by *H. pylori* VacA [88] or the enteropathogenic *E. coli* proteins EspF and Map [89,90], targeting of microbial proteins to mitochondria can directly or indirectly disrupt the mitochondrial membranes and modulate cell death. Recently, more subtle mechanisms have emerged: the *V. cholerae* secreted effector VopE acts as a mitochondria-localized GTPase-activating protein for the mitochondrial protein Miro, thereby disrupting its ability to mediate kinesin-dependent mitochondrial motility and resulting in mitochondrial fragmentation [91]. Similar strategies are used by plant pathogens. For instance, the type III secretion system (T3SS) of *Pseudomonas syringae* pv tomato DC3000 secretes Hop1N, a cysteine protease targeted to the host chloroplast, which interferes with photosynthesis by degrading a component of the photosystem, PsbQ [92]. This suppresses ROS production involved in the plant innate immune response to bacterial infection, and counteracts host programmed cell death. Other pathogens, such as *Chlamydia*, *Legionella* or *Toxoplasma* recruit mitochondria to manipulate bioenergetic and innate immune pathways [93-95]. In the case of *Toxoplasma*, the molecular basis for this phenomenon has recently been uncovered with the identification of mitochondrial association factor 1 (MAF1), a *Toxoplasma* protein that directly binds host cell mitochondria [96].

Ultimately, the subversion of organelles can result in their colonisation, as mentioned in the introduction. This has so far been essentially documented for endosymbionts, such as the bacterial inhabitants of paramecium macro- and micronuclei [97] or the intra-mitochondrial bacterial species discovered in ticks, *Candidatus Midichloria mitochondrii* [3], and intranuclear growth has also been reported for a pathogen, the spotted fever agent *Rickettsia rickettsii* [98,99].

Together, these studies pave the way to a better understanding of how bacteria take advantage of their host at the nano- and microscopic levels. However, our perception of organelle dynamics during infection remains incomplete. Functional studies by high resolution and live-cell imaging should, in the coming years, allow us to better define the mechanisms that govern the morphological and functional changes in the ER and mitochondria. They should allow us to fully appreciate the impact of bacteria on vesicle trafficking, and the key role played by the cytoskeleton in the redistribution of intracellular compartments during infection. Whether *Listeria* infection also influences other key organelles such as peroxisomes, the nucleo-cytoplasmic transport pathways, or the architecture and function of sub-compartments not delineated by a membrane, such as P-bodies or stress granules, are still open questions.

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Box 1. Outstanding Questions

1. Does the organelle response to infection impact bacterial replication beyond innate immune signalling? In particular, how does mitochondrial bioenergetics affect bacterial replication?
2. What molecules perform mitochondrial abscission in the absence of the main fission protein Drp1? Is this pathway also at work in Drp1-containing cells?
3. Does *Listeria* infection cause peroxisome restructuring, given that the mitochondrial division machinery also acts in peroxisomal division [100]?
4. Can cells keep an epigenetic memory of previous infections? The discovery that bacterial pathogens can change the pattern of histone modification on chromatin has led to the tempting hypothesis that infected cells could be imprinted with a memory of infection, independently of the well-described commitment of memory B and T lymphocytes. Research in the patho-epigenetics field still has to provide evidence that the epigenetic marks deposited during infection are long-lasting and play a role in host cell behaviour during re-infection.
5. Are the infection-induced signals from different organelles integrated? If they are, how is this achieved, and at which cellular level?

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Glossary

Chromatin: Eukaryotic DNA is condensed into an organised ribonucleoproteic assembly named chromatin. The more or less dense structure of this arrangement has several functions. During metaphase, chromatin displays its most compact form, which participates in the partition of genetic material among daughter cells. In other phases, the tightness of chromatin varies locally, on distances spanning over a few nucleosomes to a whole chromosome. The ability of chromatin to switch from an open (euchromatin) to a closed (heterochromatin) state is a potent regulator of gene expression.

Intracellular bacteria: Bacteria that invade and replicate inside host cells. They are subdivided into facultative intracellular bacteria (for example *Listeria monocytogenes*, *Salmonella Typhimurium* or *Shigella flexneri*) and obligate intracellular bacteria (for example several *Rickettsia*).

Mitochondrial membrane potential: An electrochemical gradient formed when the electron transport chain located in the inner mitochondrial membrane pumps protons from the mitochondrial matrix to the intermembrane space. Proton backflux from the intermembrane space to the matrix through the FoF1 ATPase leads to ATP synthesis.

Nucleomodulins: An emerging class of secreted bacterial effectors that enter the nucleus of the host cell, where they can affect various functions including DNA replication and repair, transcription, chromatin remodelling or RNA splicing [69].

Organelle: A cellular compartment that is separated from the cytosol by one or several membranes. Note that the definition of organelles may vary, from a stricter version only encompassing DNA-containing compartments (namely, mitochondria and plastids), to a more relaxed terminology that would include non-membrane enclosed structures visible in microscopy such as nucleoli, centrioles, stress- or P-granules. For the purpose of clarity in this review, we opted for the most-conventional definition as a membrane-enclosed intracellular compartment.

Unfolded Protein Response: A cellular quality control and defence mechanism, which is activated upon disruption of the proteostatic balance in the ER. To compensate the loss of ER function, this pathway aims at reducing the accumulation of abnormally-folded proteins, by (i) decreasing protein synthesis and translocation into the ER; (ii) stimulating the production of ER protein chaperones, which assist protein folding; (iii) promoting the degradation of unfolded proteins in the cytoplasm. If these mechanisms are ineffective, cell death is triggered [101].

Figures

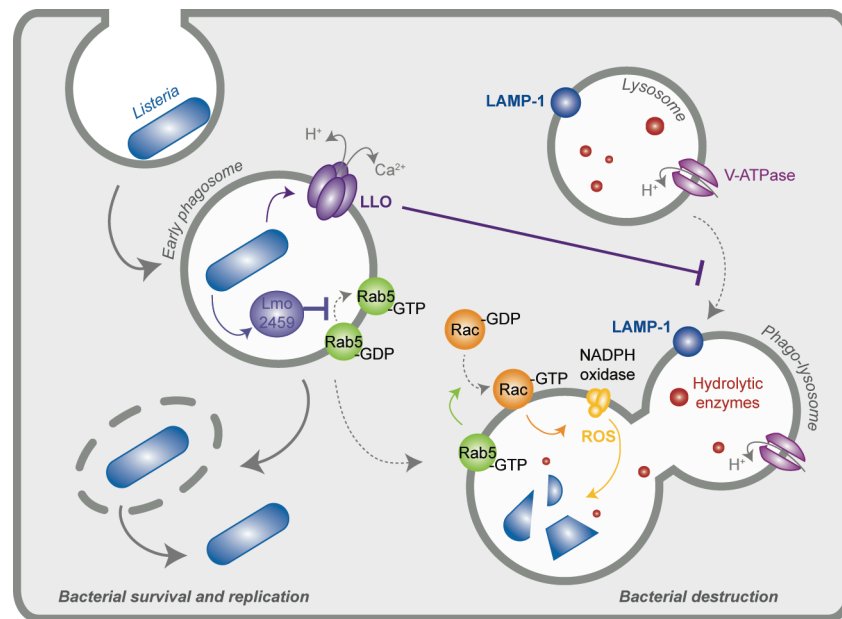


Fig. 1. Inhibition of phago-lysosomal maturation by *Listeria*. After phagocytosis by macrophages, the bacteria-containing phagosome may fuse with LAMP-1-positive lysosomes, into a phago-lysosome. ROS toxicity produced by NADPH oxidase and the action of hydrolytic enzymes, which is enhanced by the acidification of the organelle, results in bacterial killing and degradation. The secretion of LLO by *Listeria* decreases phagosomal calcium concentration and increases pH, which impede phago-lysosomal fusion. Another secreted effector, Lmo2459, blocks the maturation of the phagosome via the inhibition of Rab5.

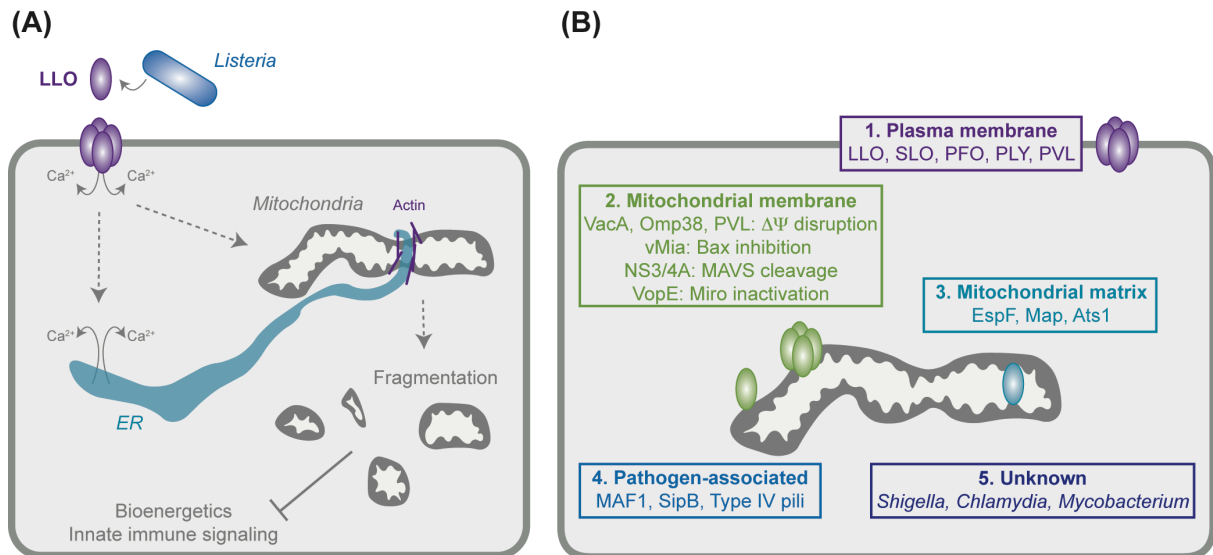


Fig. 2. Mitochondria and bacterial infections. (A) *Listeria*-induced mitochondrial fragmentation. LLO secreted by *Listeria* can form ion-permeable pores in the plasma membrane, thereby causing mitochondrial fragmentation. This fragmentation is facilitated by the ER and actin, but probably requires additional downstream factors, as it is independent of the main mitochondrial fission factor Drp1. Structural and functional disruption of the mitochondrial network causes a decrease in cellular bioenergetics and may modulate the mitochondria-based innate immune response. Although LLO has not been detected on the ER, it was shown to cause IP₃R-independent calcium release. **(B) Pathogen strategies to interfere with host cell mitochondria.** Bacterial, viral or protozoan proteins can target mitochondria in different ways. Several pore-forming toxins, such as Listeriolysin O (LLO, *Listeria monocytogenes*), Streptolysin O (SLO, *Streptococcus pyogenes*), Perfringolysin (PFO, *Clostridium perfringens*), Pneumolysin (PLY, *Streptococcus pneumoniae*) and Panton-Valentine leukocidin (PVL, *Staphylococcus aureus*) insert into the plasma membrane, thereby causing ion imbalances and activating signalling pathways that affect mitochondrial morphology and function (1). Other toxins, such as VacA (*Helicobacter pylori*), Omp38 (*Neisseria gonorrhoeae*) and PVL insert into the outer or inner mitochondrial membrane, causing a loss in the mitochondrial membrane potential $\Delta\Psi$ (2). The viral proteins vMia (cytomegalovirus) and NS3/4A (Hepatitis C) localize to the outer membrane and respectively induce Bax inhibition along with mitochondrial fragmentation, and MAVS cleavage. *Vibrio cholerae* VopE is a GTPase activating protein that inactivates Miro at mitochondria, inducing mitochondrial fragmentation. The secreted effectors EspF, Map (enteropathogenic *Escherichia coli*) and Ats1 (*Anaplasma phagocytophilum*) are imported into mitochondria and act “from within” to interfere with mitochondrial apoptosis pathways (3). Yet other effectors act on mitochondria while remaining pathogen-associated (4): *Toxoplasma gondii* MAF1 recruits mitochondria to the parasitophorous vacuole; *Salmonella enterica* SipB induces the formation of mitochondria-derived membrane structures; *Neisseria gonorrhoeae* type IV pili inhibit apoptosis. Other microbial effectors either remain unknown or act on mitochondria from unknown locations that include other organelles or the cytoplasm (5).

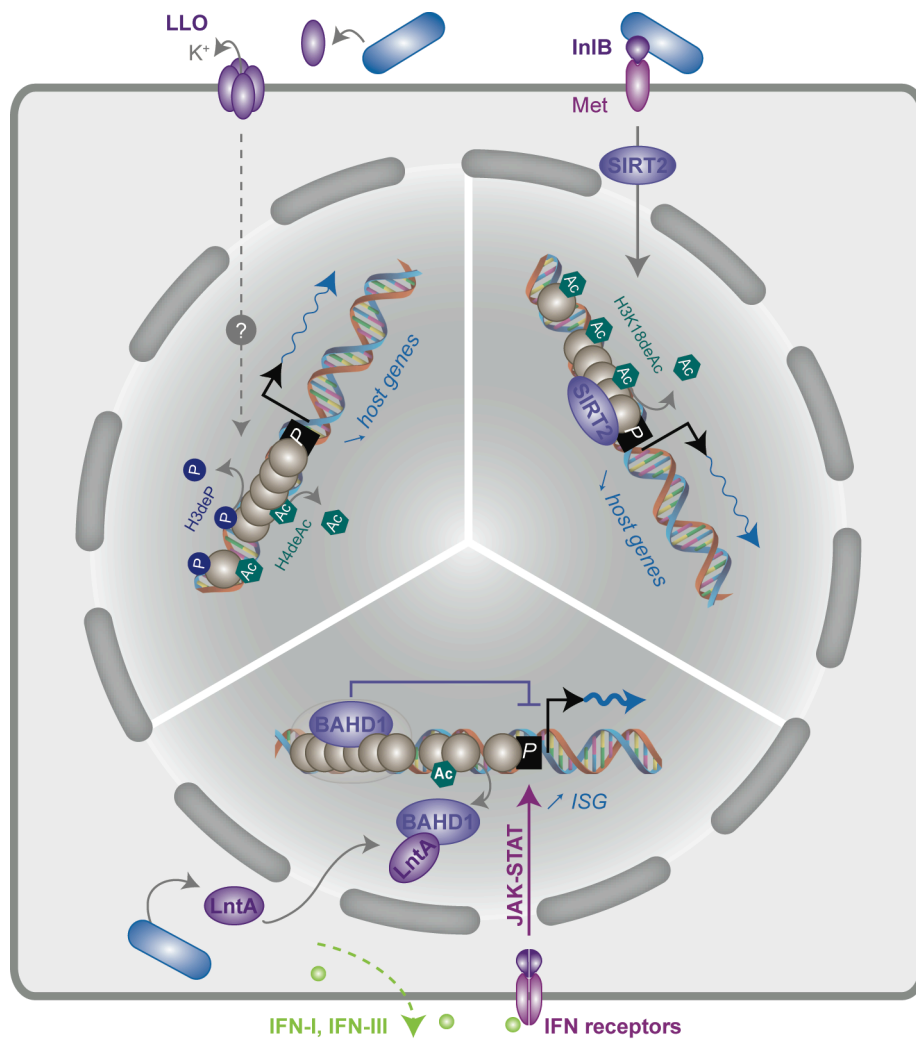


Fig. 3. Subversion of nuclear functions by *Listeria*. *Left.* By secreting the pore-forming toxin LLO, *Listeria* generates potassium efflux from the host-cell, which promotes a global deacetylation of histone H4 and dephosphorylation of Histone H3 on serine 10. This results in the down-regulation of a subset of host genes. *Right.* Binding of *Listeria* InIB to its receptor Met at the host cell membrane triggers, via a PI3K/Akt-dependent signalling cascade, the translocation of the lysine deacetylase SIRT2 to the nucleus. SIRT2 associates with chromatin at the promoter of various host genes, triggers the deacetylation of lysine 18 on H3, and thereby represses these genes. *Bottom.* In response to *Listeria* infection of epithelial cells, type I and III interferons are produced. However, the expression of ISGs is repressed downstream of JAK/STAT signalling, due to the condensation of a repressive complex at their promoter by the host chromatin component BAHD1. Under specific conditions, *Listeria* secretes the nucleomodulin LntA, which translocates to the nucleus, binds directly with BAHD1 and inhibits its function, resulting in an enhanced expression of ISGs.