Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis

Lilliana Radoshevich[1–4] *and Pascale Cossart*[1–3]

Abstract | *Listeria monocytogenes* is a food-borne pathogen responsible for a disease called listeriosis, which is potentially lethal in immunocompromised individuals. This bacterium, first used as a model to study cell-mediated immunity, has emerged over the past 20 years as a paradigm in infection biology, cell biology and fundamental microbiology. In this Review, we highlight recent advances in the understanding of human listeriosis and *L. monocytogenes* biology. We describe unsuspected modes of hijacking host cell biology, ranging from changes in organelle morphology to direct effects on host transcription via a new class of bacterial effectors called nucleomodulins. We then discuss advances in understanding infection *in vivo*, including the discovery of tissue-specific virulence factors and the 'arms race' among bacteria competing for a niche in the microbiota. Finally, we describe the complexity of bacterial regulation and physiology, incorporating new insights into the mechanisms of action of a series of riboregulators that are critical for efficient metabolic regulation, antibiotic resistance and interspecies competition.

[1]*Institut Pasteur, Unité des Interactions Bactéries-Cellules, Département de Biologie Cellulaire et Infection, F-75015 Paris, France.*
[2]*Inserm, U604, F-75015 Paris, France.*
[3]*French National Institute for Agricultural Research (INRA), Unité sous-contrat 2020, F-75015 Paris, France.*
[4]*Present address: Department of Microbiology and Immunology, University of Iowa Carver College of Medicine, Iowa City, Iowa 52242, USA.*
lilliana-radoshevich@uiowa.edu; pascale.cossart@pasteur.fr

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Listeria monocytogenes is a Gram-positive bacterium first described in 1926 during an outbreak that affected rabbits and guinea pigs[1]. It was recognized in the 1970s as the aetiological agent of a human disease and identified in the 1980s[2] as a food-borne pathogen. Although the number of infections per year is moderately low (approximately 23,150 cases were estimated worldwide in 2010), the mortality among infected individuals is very high (20–30%)[3]. After ingestion of highly contaminated food (up to ~10^9 bacteria), most individuals experience mild to severe gastroenteritis[2]. By contrast, in the case of children, elderly individuals, immunocompromised individuals and pregnant women, even low levels of food contamination (~10^2–10^4 bacteria) can lead to bacterial sepsis, subsequent bacterial meningitis and/or infection of the fetus, resulting in abortion or complications to pregnancy[4,5] (FIG. 1).

Until recently, the genus *Listeria* was thought to contain only eight species and two subspecies. However, the number of identified species has now increased to 17 (REF. 6) (BOX 1). Two species, *Listeria monocytogenes* and *Listeria ivanovii,* are pathogenic to humans and ruminants, respectively. All *Listeria* spp. are rod-shaped facultative anaerobes that can grow at low temperatures and are quite resistant to environmental stresses, such as low pH and high salt concentrations, features that make *L. monocytogenes* a major concern for the food industry[7,8]. *Listeria* spp. can thrive in various environments and are often isolated from water, soil and detritus. An arsenal of regulatory factors allows the bacterium to oscillate between survival in the environment and infection of a mammalian host[9]. Thanks to its adaptability, its capacity to cross various host barriers and its unique intracellular lifestyle, *L. monocytogenes* has come to the forefront as a model in the study of bacterial regulation, host-pathogen interactions and, more recently, interactions with the gut microbiome. In this Review, we discuss recent advances in *L. monocytogenes* biology, which have unveiled new concepts in bacterial regulation, epigenetic modification and the induction of innate and adaptive immune responses (animated model of *L. monocytogenes* infection biology).

Cell biology of the infection process

Upon ingestion of contaminated food by the host, *L. monocytogenes* encounters the intestinal epithelium, traverses the intestinal epithelial barrier into the lamina propria and then disseminates via the lymph and blood towards its target organs, the liver and spleen[10]. It can then cross the blood–brain barrier in

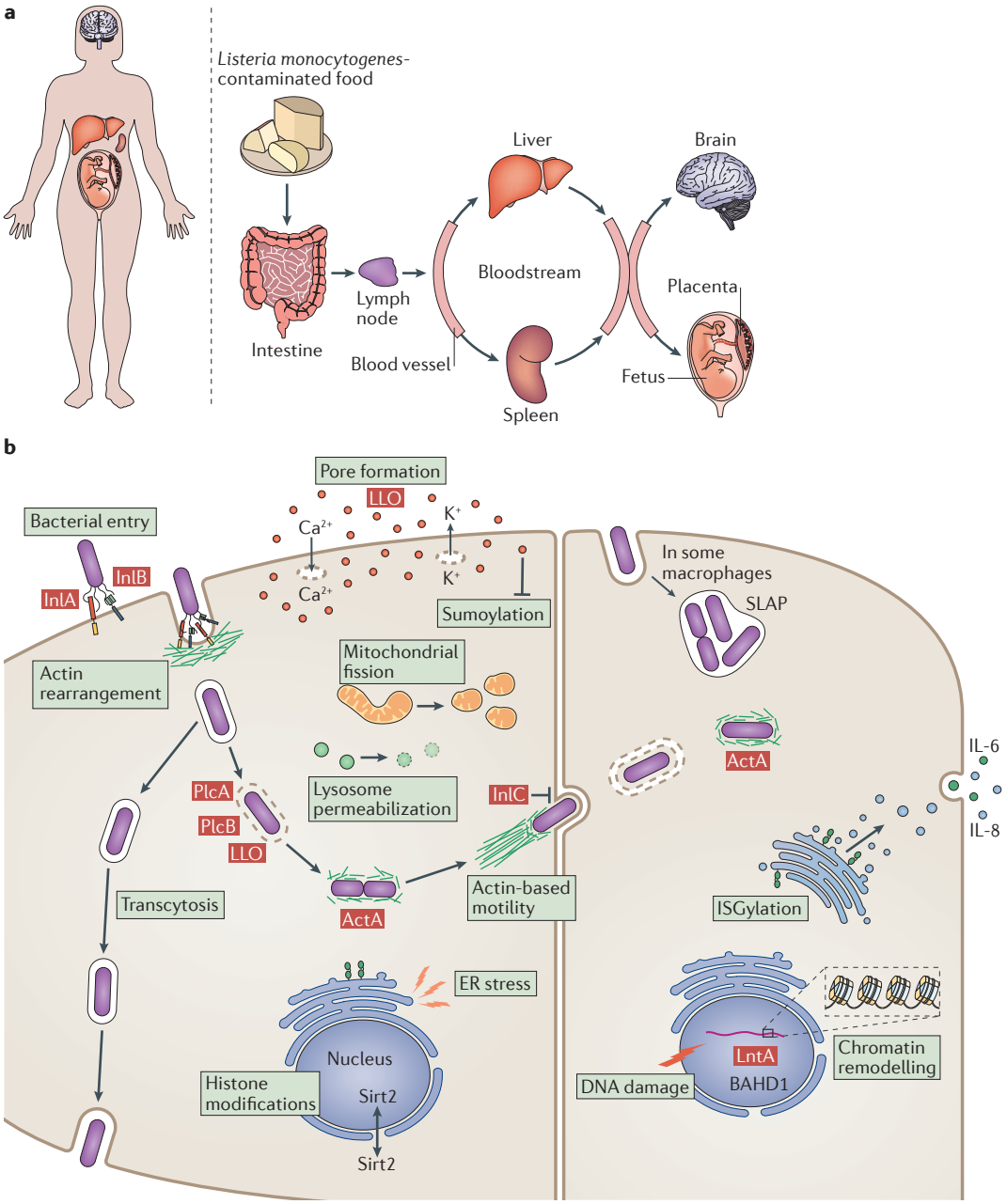


Figure 1 | **Overview of *Listeria monocytogenes* infection. a** | Schematic of *Listeria monocytogenes* infection of a human host. After ingestion of contaminated food, *L. monocytogenes* can traverse the intestinal barrier and spread into the bloodstream through the lymph nodes to disseminate to target tissues, such as the liver and spleen. In immunocompromised individuals, *L. monocytogenes* can cross the blood–brain barrier or fetoplacental barrier and cause potentially fatal meningitis, sepsis, premature birth or abortion. **b** | *L. monocytogenes* enters non-phagocytic cells, such as epithelial cells, through receptor-mediated endocytosis, and in most cases, it escapes from the vacuole. In goblet cells, it can transcytose across the cell within a vacuole, and in some macrophages, it can replicate in spacious *Listeria*-containing phagosomes (SLAPs). Upon vacuolar escape, *L. monocytogenes* subsequently polymerizes actin and can spread from cell to cell. *L. monocytogenes* infection has a plethora of effects on the cell through the activity of potent virulence factors. Listeriolysin O (LLO), phospholipase A (PlcA) and PlcB mediate vacuolar escape. LLO also leads to changes in histone modification, desumoylation, mitochondrial fission, endoplasmic reticulum (ER) stress and lysosomal permeabilization, all of which can occur from the pore-forming activity of extracellular LLO. *Listeria* nuclear targeted protein A (LntA) interacts with the Bromo adjacent homology domain-containing 1 protein (BAHD1) complex to derepress interferon-stimulated genes (ISGs), and NAD-dependent protein deacetylase sirtuin 2 (SIRT2) shuttles into the nucleus to deacetylate histone 3 at lysine 18, leading to changes in chromatin packing that alter downstream gene expression. Infection also leads to DNA damage, and the host cell combats infection by upregulating a number of antibacterial effectors, for example, ISG15 and the process of modification by ISG15 called ISGylation, which modulates cytokine secretion by covalent modification of ER and Golgi proteins. ActA, actin assembly-inducing protein; IL, interleukin; Inl, internalin.

Actin nucleation
The assemblyof monomeric actin into filaments by actin nucleators, which can result in branched or linear actin filaments depending on the actin nucleator.

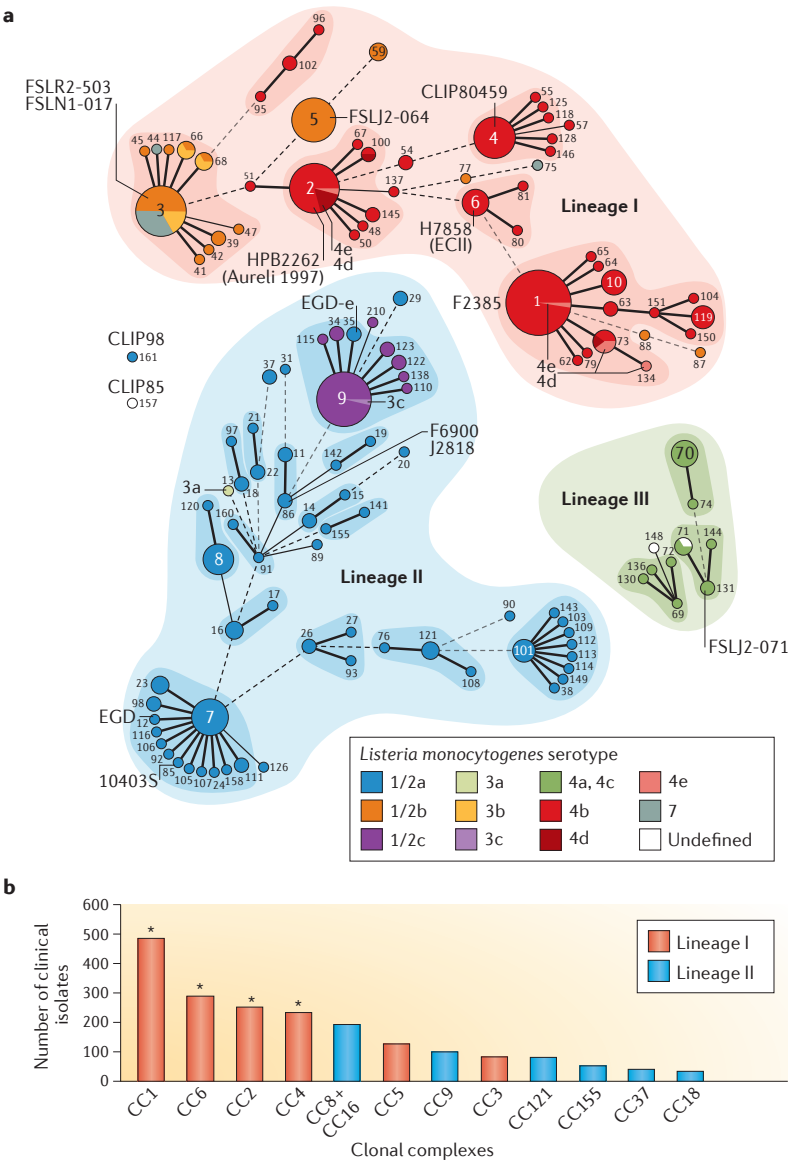
Actin-based motility
Listeria monocytogenes-mediated motility co-opts cellular actin nucleators to form bundles of actin that propel the bacterium within the cell and allow it to spread from one cell to another.

immunocompromised individuals or the fetoplacental barrier in pregnant women[11] (FIG. 1a). Following entry into non-phagocytic cells such as epithelial cells, or uptake by phagocytic cells, *L. monocytogenes* is internalized into the vacuole[12]. This first step requires actin nucleation and polymerization, which leads to cytoskeletal rearrangement. In most cases, the bacterium then escapes from the vacuole by physically disrupting the vacuolar membrane through the activity of potent virulence factors, which will be discussed below. *L. monocytogenes* can survive and divide in the cytosol of host cells and thereby alters a plethora of host cell processes and also organelles. *L. monocytogenes* can also spread from one cell to another by co-opting actin-based motility[13] (FIG. 1b). While the bacterial virulence factors required for entry, phagosomal escape and cell-to-cell spread have been well characterized, a number of other factors have been recently reported that hijack cellular processes and, in some cases, reach the eukaryotic nucleus, inducing epigenetic changes that influence gene expression[14].

Entry into cells. *L. monocytogenes* can be internalized in both phagocytic and non-phagocytic cells. In contrast to entry into phagocytic cells, which is actively mediated by the phagocyte, entry into non-phagocytic cells is initiated by bacteria that co-opt the cellular receptor-mediated endocytosis machinery. Indeed, one of the hallmarks of *L. monocytogenes* infection is the remarkable capacity of the bacterium to enter non-phagocytic cells (FIG. 2). Entry is perhaps the most extensively characterized step of *L. monocytogenes* infection. Internalin A (InlA) and InlB, two members of a family of 25 proteins called internalins, bind to eukaryotic cell membrane receptors, E-cadherin and Met, the receptor of the hepatocyte growth factor (HGF),

Box 1 | *Listeria* phylogeny

Currently, there are 17 identified *Listeria* species, of which 2 are pathogenic (*Listeria monocytogenes* and *Listeria ivanovii*) and 6 share sufficient genotypic and phenotypic similarities to be considered *Listeria* sensu stricto[6,119]. *Listeria* strains were historically characterized by serotyping. There are 13 serotypes of *Listeria* that have been classified into 4 evolutionary lineages and 63 clonal complexes (CCs) by multilocus sequence typing. **a** | Three of these lineages are depicted. **b** | Twelve of the CCs account for nearly 80% of isolates identified in a recent prospective study[120]. Notably, clinical isolates were enriched in specific CCs. These clinical CCs were primarily from lineage I and were much more virulent in an animal model of listeriosis[120]. Furthermore, core genome sequencing of these CCs revealed putative virulence loci among these strains that could potentially explain central nervous system tropism or materno–neonatal tropism[120]. Recent quantification of the evolutionary rate of *Listeria* estimated that the origin of the major sublineages was approximately 50–150 years ago. The study also reported international dissemination of sublineages. These findings led to the hypothesis that *L. monocytogenes* may have emerged as a pathogen relatively recently and potentially in association with movement of humans, animals and food[121]. Historically, three laboratory strains from lineage II have been used to study *L. monocytogenes* biology (EGD, EGD-e and 10403s, highlighted in part **a** of the figure). A recent study compared genomic and phenotypic aspects of these widely used strains[91] and found differences, such as various phage integration sites. It is likely that parallel work on lineage I strains will prove to be similarly informative and potentially clinically relevant.



*Association with food or clinical origin (χ^2 test): $P < 0.0001$. Figure part **a** is adapted with permission from REF. 122, PLoS; and the American Society for Microbiology (REF. 91): Becavin, C. *et al.* Comparison of widely used Listeria monocytogenes strains EGD, 10403S, and EGD-e highlights genomic variations underlying differences in pathogenicity. *mBio* **5**, e00969-14 (2014) http://dx.doi.org/10.1128/mBio.00969-14. Figure part **b** is from REF. 120, Macmillan Publishers Limited.

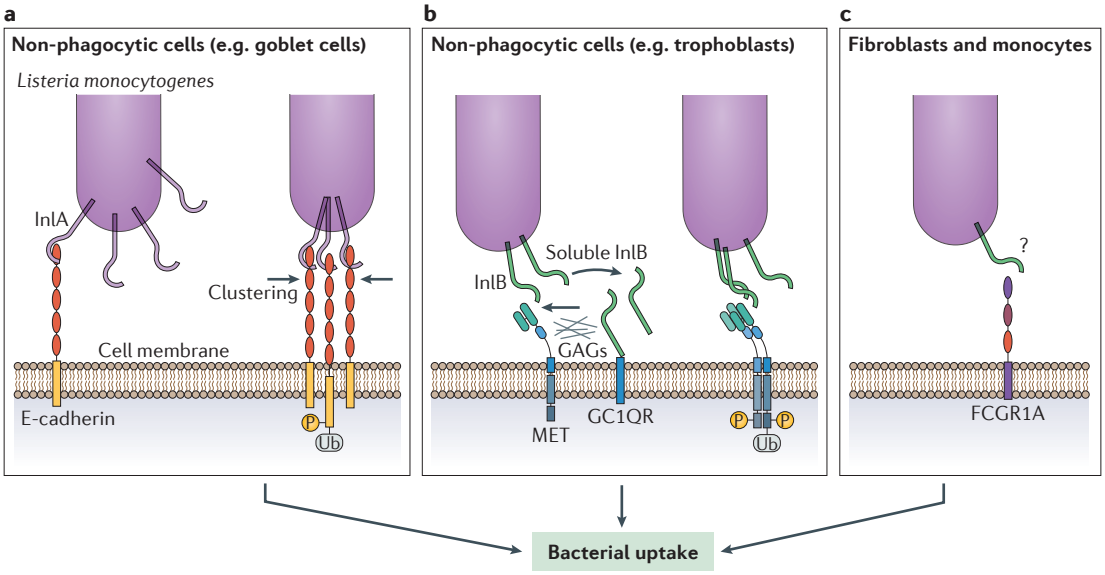


Figure 2 | **Entry of *Listeria monocytogenes* into cells. a** | In non-phagocytic cells, such as goblet cells, *Listeria monocytogenes* internalin-A (InlA) binds E-cadherin, which in turn leads to receptor clustering, E-cadherin phosphorylation (P) and ubiquitylation (Ub), resulting in bacterial uptake by the cell. **b** | In non-phagocytic cells such as trophoblasts, *L. monocytogenes* InlB binds the hepatocyte growth factor receptor (Met) and induces its phosphorylation and ubiquitylation and subsequent receptor-mediated endocytosis of the bacteria. In addition, binding of soluble InlB to the complement component 1 Q subcomponent-binding protein (C1QBP; also known as GC1QR) and to cell-surface glycosaminoglycans (GAGs), which facilitates receptor clustering, helps to speed up bacterial uptake. **c** | Finally, a yet-unknown bacterial factor binds the high affinity immunoglobulin gamma Fc receptor I (FCGR1A) on monocytes and fibroblasts, thus leading to bacterial uptake independently of opsonization.

respectively, thereby inducing bacterial uptake through receptor-mediated endocytosis[15]. Internalins can be anchored to the cell wall, associated with the cell wall or secreted and together mediate a variety of virulence-related functions[16]. For example, in addition to InlA and InlB, InlC affects the rigidity of the cytoskeleton and innate immune signalling, InlP mediates placental invasion and InlJ is expressed solely *in vivo*, though its cellular receptor and tissue tropism remain to be identified[17].

Recently, several large-scale approaches have led to novel insights into bacterial entry. A genome-wide small interfering RNA (siRNA) screen for host factors that affect *L. monocytogenes* infection unlocked distinct roles for individual proteins from cellular complexes or protein families, which were previously thought to act in concert[18]. The most striking result from this study concerned the actin-nucleating complex actin-related protein 2/3 (ARP2/3). The canonical complex is composed of seven subunits: ARP2, ARP3 and ARPC1–5, which together mediate actin nucleation and branching. This study revealed that distinct ARP2/3 complexes, composed of different protein subsets, are required either for actin nucleation at the cell surface to facilitate bacterial entry or for actin comet formation, which is necessary for cell-to-cell spread, thus challenging the long-held tenet of a universal ARP2/3 complex. Another genome-wide siRNA screen in *Drosophila* S2 cells sought host factors that alter infection and revealed that inhibition of the cytoskeleton-remodelling Rho-associated protein kinases (ROCKs) could facilitate bacterial entry[19]. Subsequent work identified InlF as required for increased entry following ROCK inhibition through an as-yet-unknown species-specific mechanism[20]. A third large-scale approach used a fluorescence-based gain-of-function screen to dissect the role of specific interferon-stimulated genes (ISGs) that are induced during *L. monocytogenes* infection[21]. By infecting cells that stably express 350 ISGs and assessing bacterial load, the authors found that the high affinity immunoglobulin gamma Fc receptor I (FCGR1A; also known as CD64) can act as an additional receptor for *L. monocytogenes* in both fibroblasts and monocytes, highlighting a mechanism for bacterial entry that is distinct from InlA-mediated entry or InlB-mediated entry. During uptake of extracellular bacteria by phagocytes, immunoglobulin receptors mediate bacterial engulfment through the process of opsonization; however, FCGR1A-mediated *L. monocytogenes* uptake does not require this pathway. Dissecting the mechanism of endocytosis, understanding the *in vivo* relevance of this new internalization pathway and identifying the bacterial effector to which FCGR1A binds will further elucidate its role during infection.

Escape from or residence in the vacuole. Once the bacterium is internalized inside the vacuole, it uses listeriolysin O (LLO) and two phospholipases, phospholipase A (PlcA; also known as 1-phosphatidylinositol phosphodiesterase) and PlcB, for vacuolar rupture and escape, which are crucial steps in *L. monocytogenes* pathogenesis (FIG. 1b). Two additional components

Receptor-mediated endocytosis
Cellular uptake of host surface receptors to regulate growth factor signalling or receptor turnover; the process requires monoubiquitylation of the receptor, clathrin and actin.

Internalin
A *Listeria monocytogenes* protein characterized by leucine-rich repeat domains that can be anchored to the bacterial cell wall by a sorting motif or secreted.

Vacuolar rupture
Vacuolar damage (or phagosomal damage in phagocytic cells) by bacterial virulence factors that allow bacterial escape into the cytosol.

involved in vacuolar escape were recently identified. In the context of bacterial communities, small molecules can serve as signals for the bacteria to act in concert (for example, to promote biofilm formation) in a process called quorum sensing. Intriguingly, the vacuole can physically confine bacterial signalling molecules, thus concentrating them to effectively mimic a larger bacterial population. In non-phagocytic cells, a PrfA-dependent lipoprotein called peptide pheromone-encoding lipoprotein A (PplA) is upregulated, and upon secretion of the lipoprotein from the bacterium, the N-terminal pPplA peptide is released and promotes vacuolar escape[22]. The pPplA peptide does not affect the efficiency of perforation of the vacuole but instead leads to altered signalling and secretion of protein translocase subunit SecA2-dependent substrates, which likely contribute to vacuolar escape in an as-yet-unknown mechanism. In addition, a compelling paper revealed that in professional phagocytes, phage excision from the bacterial genome restores the activity of a competence gene, named *comK*, and strikingly favours vacuolar escape in macrophages[23]. Indeed, in some *Listeria* strains, *comK* is interrupted by the insertion of a DNA bacteriophage, and when the phage is excised, during bacterial intracellular growth, its downstream targets (*com* genes) are upregulated. Moreover, when bacteria are cytosolic, the phage DNA is reinserted in the chromosome and transcription of the *com* genes is inhibited. The signals that mediate phage excision are still unknown[24]. In addition, ComG, a type II protein secretion pseudopilus, and ComEC, a membrane channel, were shown to be required for bacterial phagosomal escape, leading to the hypothesis that their structures contribute to the physical disruption of the phagosome[24].

Mice with severe combined immune deficiency cannot clear *L. monocytogenes* infection, and in this context, wild-type bacteria do not escape the phagosome but rather replicate slowly in spacious *Listeria*-containing phagosomes (SLAPs)[25]. Formation of SLAPs occurs in macrophages and requires LC3-mediated phagocytosis as well as intermediate levels of LLO expression, which is sufficient to interfere with the pH gradient required for acidification of the phagosome but not to promote phagosomal rupture[26].

Furthermore, during bacterial crossing of the intestinal barrier *in vivo*, *L. monocytogenes* remains in the internalization vacuole and very rapidly transcytoses through goblet cells[27]. Therefore, in certain cell types, this pathogen could be considered both a vacuolar and a cytosolic bacterium.

Changes in organelle morphology and function. *L. monocytogenes* can survive and divide within the cytosol of the host cell and induce changes in the morphology of host cell organelles, thereby altering their function to promote infection. Although the first reported function of LLO was pore formation leading to phagosomal rupture, several recent studies have highlighted additional functions of LLO at the plasma membrane before entry and potentially within the cell after internalization[28]. Upon bacterial infection, LLO causes a dramatic alteration in mitochondrial morphology and function that benefits the pathogen by an as-yet-unknown mechanism. Indeed, mitochondria become small and rounded[29], and if this process is experimentally abrogated, bacteria cannot divide as efficiently. Moreover, LLO induces a non-canonical mitochondrial fission process that is independent of the dynamin-related protein 1 (DRP1)[30]. The mitochondria are constricted following contact with the endoplasmic reticulum (ER) at points of fission, and actin polymerization is required to potentially generate the necessary force for fission in the absence of Drp1 (REF. 30).

Mitochondria are not the only organelles to be affected by this potent toxin; a recent report has shown that LLO also affects the ER[31]. When the protein folding demand in the ER exceeds its capacity, the cell senses this state of ER stress and upregulates the unfolded protein response (UPR), which blocks translation and protein import into the ER, upregulates ER chaperones to expand the ER and increases ER-associated protein degradation to remove misfolded proteins. Through an unknown mechanism, infection increases ER stress, and this effect can be mimicked by *in vitro* LLO addition. Induction of ER stress before infection in turn reduces bacterial replication within cells, a finding that reflects the observation that animals with an attenuated UPR are more susceptible to bacterial infection[32].

Finally, lysosomes are also altered by LLO. Following infection, lysosomal membrane integrity is compromised[33], a phenomenon accompanied by release of active cathepsins into the cytosol. Although the function of this process is still unknown, future areas of investigation should shed light on how it affects immune signalling, bacterial load and lysosomal homeostasis.

Transcriptional and epigenetic regulation. Upon infection, *L. monocytogenes* can manipulate host cell transcription and induce epigenetic modifications. Recent work has highlighted a new class of bacterial virulence factors that can either directly or indirectly modulate gene expression to tune the innate immune response to invading pathogens. These factors, termed nucleomodulins, are secreted from the bacterium into the cell cytoplasm, and from there, they can reach the nucleus to exert their dedicated functions[14]. The first *L. monocytogenes* nucleomodulin that has been identified is called *Listeria* nuclear targeted protein A (LntA). LntA interacts with the chromatin repressing bromo adjacent homology domain-containing 1 protein (BAHD1) to derepress specific ISGs in a type III interferon-dependent manner, and this paradoxically benefits the pathogen[34]. The LntA–BAHD1 interaction is direct, and abrogation of this interaction inhibits the effect of LntA on ISGs and its colocalization with BAHD1 in the nucleus[35,36]. *L. monocytogenes* also secretes cyclic di-AMP and releases other pathogen-associated molecular patterns (PAMPs) during infection, both of which activate the cellular innate immune response (see BOX 2 for details)[37–39].

Phage excision
The active process of removal of DNA from a lysogenic (non-lytic) bacteriophage that was previously integrated into the bacterial genome.

LC3-mediated phagocytosis
Phagocytosis in which the autophagy microtubule-associated protein light-chain 3 (LC3) is conjugated to the lipid phosphatidylethanolamine on the inside of the plasma membrane.

Mitochondrial fission
Mitochondrial division mediated by dedicated cellular factors called dynamin-related protein 1 (DRP1 and mitochondrial fission factor (Mff).

Unfolded protein response
(UPR.) When the protein folding demand of the endoplasmic reticulum (ER) exceeds its capacity, this response upregulates chaperones, blocks translation into the ER and increases ER folding capacity.

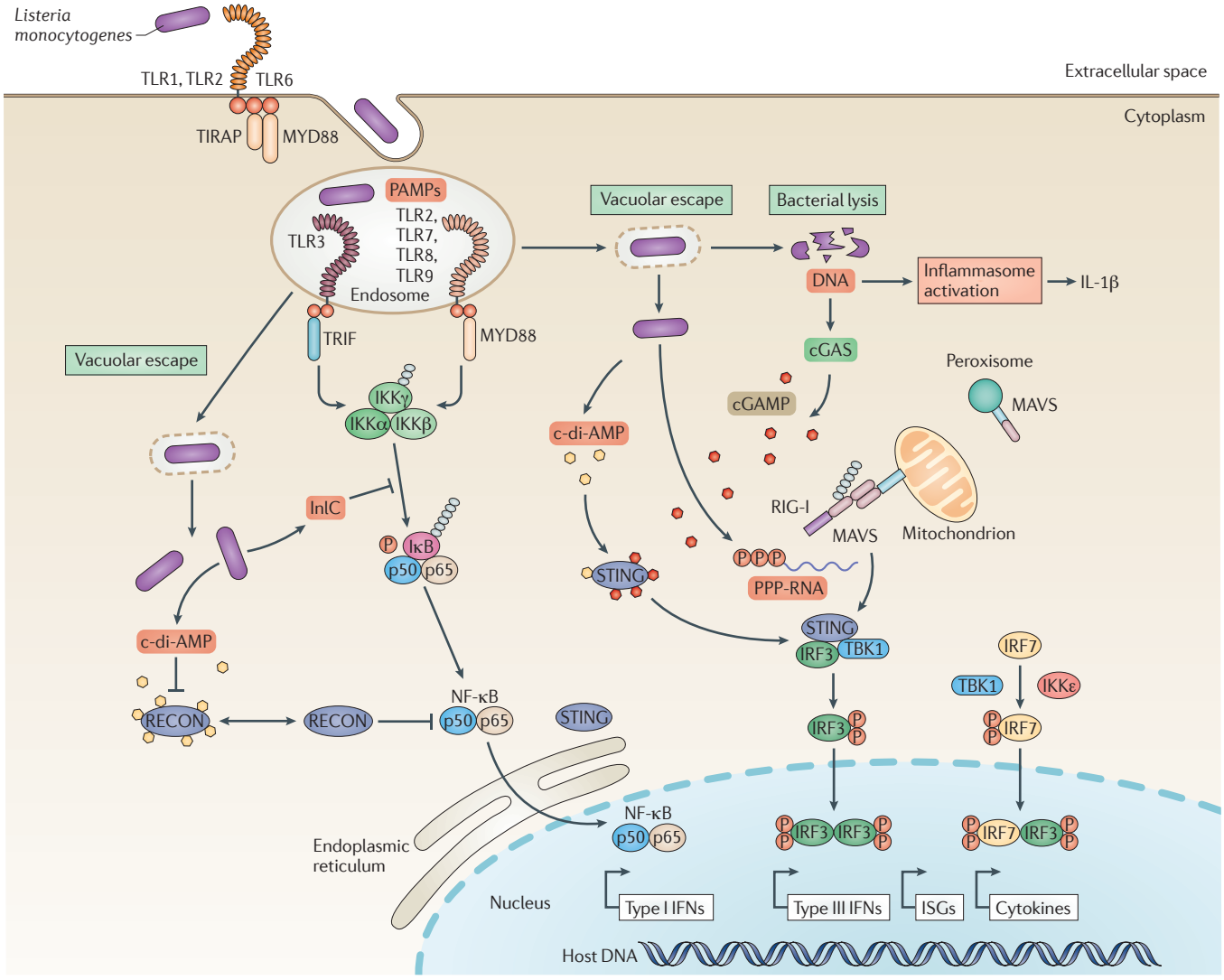
Cathepsins
A class of proteases that degrade other proteins and are typically activated by the acidic conditions in the lysosome.

Nucleomodulins
A class of bacterial virulence factors that are expressed in the cytoplasm and travel to the nucleus where they can affect host transcription.

Bromo adjacent homology domain-containing 1 protein
(BAHD1). A protein that is part of a transcriptional repression complex that affects the expression of interferon-stimulated genes following *Listeria monocytogenes* infection.

Box 2 | **Innate immune sensing of *Listeria monocytogenes***

Upon infection, *Listeria monocytogenes* pathogen-associated molecular patterns (PAMPs) are sensed on the surface of cells by Toll-like receptors (TLRs). TLRs also sense bacteria in the phagosome, leading to nuclear factor-κB (NF-κB) activation. Bacterial lysis in the cytosol activates the inflammasome as well as cyclic GMP-AMP synthase (cGAS), which produces a host-specific cyclic dinucleotide (CDN) called cyclic GMP-AMP (cGAMP) that also activates stimulator of interferon genes protein (STING). Bacterial 5′-triphosphate RNA (indicated as PPP-RNA in the figure) is either released passively or potentially secreted actively by the pathogen and sensed by retinoic acid-inducible gene I protein (RIGI), leading to activation of mitochondrial forms or peroxisomal forms of mitochondrial antiviral-signalling protein (MAVS)[123,124]. These various pathways synergize to upregulate interferons, cytokines and other antibacterial effectors, such as interferon-stimulated genes (ISGs). As an example, expression of ubiquitin-like protein ISG15 was found to be induced in an early, interferon-independent manner[125] by the cytosolic surveillance pathway (including STING, serine/threonine-protein kinase TBK1, interferon regulatory factor 3 (IRF3) and IRF7) after sensing of bacterial DNA. *L. monocytogenes* secretes a small dinucleotide called cyclic di-AMP (c-di-AMP) that can directly activate STING and thereby increase interferon production in macrophages[126]. While counterintuitive, activation of innate immune pathways through c-di-AMP negatively affects the activation of T cell-mediated immunity and therefore reduces clearance of the pathogen upon secondary exposure[127]. As the affinity of STING for c-di-AMP was weaker than for host CDNs, it raised the question of whether there were other potential cellular targets of c-di-AMP. By use of affinity purification of *L. monocytogenes* c-di-AMP with lysates from various tissues, c-di-AMP was shown to bind to a liver enzyme called aldo-keto reductase family 1 member C13 (AKR1C13; also known as RECON)[128]. Additionally, c-di-AMP bound to RECON with much higher affinity than to STING, and RECON could act as a sink for CDNs to reduce STING signalling. During infection, TLR1, TLR2 and nucleotide-binding oligomerization domain-containing protein 1 (NOD1) are activated by *L. monocytogenes* PAMPs, which synergize to stimulate NF-κB signalling and mount an antibacterial response. Indeed, RECON negatively regulates NF-κB signalling, but upon binding to bacterial CDNs, it is inactivated, and antibacterial nitric oxide production and NF-κB signalling are stimulated. In addition, RECON binding to CDNs required the reductase activity of the enzyme. This was the first report of an enzymatic pattern recognition receptor (PRR) for bacterial cyclic dinucleotides that dramatically alters innate immune signalling following *L. monocytogenes* infection. The concept of a PRR that can modulate downstream responses through its enzymatic activity on an as-yet-unknown substrate is compelling, as multiple inputs from eukaryotic and bacterial cells could be integrated in order to generate and tune the appropriate downstream immune response. IκB, NF-κB inhibitor; IFN, interferon; IKKα, inhibitor of nuclear factor κB kinase-α; IL-1β, interleukin 1β; InlC, internalin C; MYD88, myeloid differentiation primary response protein; P, phosphate; TIRAP, Toll/interleukin-1 receptor domain-containing adapter protein; TRIF, TIR domain-containing adapter molecule 1.



In addition to direct effects on eukaryotic transcription, infection with *L. monocytogenes* can also induce changes in histone post-translational modifications (PTMs)[40,41], which are known to control chromatin packing in the nucleus, thereby altering the access of transcription factors to specific genetic regions. For example, upon infection, an NAD-dependent deacetylase called NAD-dependent protein deacetylase sirtuin 2 (SIRT2) shuttles to the nucleus and mediates deacetylation of histone 3 at lysine 18 (H3K18)[42]. This event is independent of LLO but dependent on the interaction of InlB with Met and leads to the repression of a number of genes, including genes for transcription factors, such as the bone morphogenetic protein SMAD1 (a combination of the names of the *Caenorhabditis elegans* SMA protein and the *Drosophila melanogaster* protein mothers against decapentaplegic 1 (MAD1)) and the forkhead box protein M1 (FOXM1). SIRT2-deficient animals show dramatically decreased bacterial loads in the liver and the spleen[42], revealing that *L. monocytogenes* requires SIRT2 activity for virulence.

Not surprisingly, as LLO is so potent, there are also effects on histone modifications and other nuclear processes that are dependent on LLO. Indeed, LLO leads to degradation of the human telomerase reverse transcriptase (TERT), an enzyme that maintains chromosomal integrity, and this is required for infection[43]. In addition, to promote infection, LLO mediates the degradation of the DNA damage sensor meiotic recombination 11 homologue 1 (MRE11), thus altering the DNA damage response that is elicited upon infection[44]. Another study showed that the DNA damage that is induced by infection can also favour it. Indeed, the authors propose that DNA damage leads to the activation of DNA damage checkpoints, thereby extending the cell cycle. Therefore, by using this strategy, the pathogen creates a favourable environment for its own proliferation in the host cytoplasm[45].

L. monocytogenes can affect other PTMs during host cell infection. Receptor-mediated endocytosis is generally triggered by phosphorylation and ubiquitylation of the cytosolic part of a receptor. *L. monocytogenes* co-opts this process by mimicking receptor-engagement, leading to the phosphorylation and ubiquitylation of E-cadherin and the Met receptor to promote its internalization by using the clathrin machinery of the host cell[46,47]. Phosphorylation of clathrin precedes actin recruitment at the entry site, and interestingly, the same machinery co-opted during infection is required for cell–cell adhesion[48,49]. Infection by *L. monocytogenes* also leads to the degradation of SUMO-conjugating enzyme UBC9, the E2 enzyme required for sumoylation of host targets, thereby provoking a global reduction in sumoylated proteins, especially in the nucleus[50,51]. One important desumoylated target is the promyelocytic leukaemia protein (PML)[52]. PML is critical for the cellular oxidative stress response, and its deletion results in extreme susceptibility to *L. monocytogenes* infection both *in vitro* and *in vivo*. Intriguingly, desumoylation of PML seems to act as a sensor of pore formation and results in the expression of antibacterial cytokines and transcription factors to help the cell respond to bacterial pathogens that produce pore-forming toxins.

Intracellular and intercellular motility. Another hallmark of the intracellular lifestyle of *L. monocytogenes* is the capacity of the bacterium to polymerize actin and spread from cell to cell. *L. monocytogenes* expresses a surface-anchored virulence factor called actin assembly-inducing protein (ActA) that is greatly induced in the host cytoplasm and interacts with the ARP2/3 complex to mediate actin polymerization and to generate sufficient force to spread from one cell to another[53]. Recent advances in cryo-electron tomography have allowed in-depth visualization of the three-dimensional architecture of actin comet tails[54]. In addition to the ARP2/3 complex, the genome-wide siRNA screen mentioned above highlighted the importance of formins during infection[18]. Formins can also induce actin nucleation and are regulated by interactions with RHO-family GTPases[55]. A second report found distinct diaphanous formins to be required for the formation of actin comet tails and maintenance of the stability and length of actin protrusions[56]. Cell division protein 42 homologue (CDC42), a RHO-family GTPase, can indirectly affect infection by maintaining cell rigidity in polarized cells and was previously shown to be thwarted by InlC to increase cell-to-cell spread[57,58]. This phase of infection exploits the process of efferocytosis in the neighbouring cell[59]. LLO mediates local membrane damage in the protrusion, which leads to surface presentation of the eukaryotic inner membrane leaflet lipid phosphatidylserine. The T cell immunoglobulin and mucin domain-containing protein 4 (TIM4) receptor on macrophages subsequently mediates the uptake of these phosphatidylserine-positive protrusions, enabling cell-to-cell spread both *in vitro* and *in vivo*[59].

It has long been appreciated that infection of type I interferon receptor 1 (IFNAR1)-deficient animals results in decreased bacterial load[38,60–63]. A recent study found that this phenotype was partially due to a lack of cell-to-cell spread both *in vitro* in macrophages and *in vivo*[64], which was associated with a lack of polarization of ActA on the surface of the bacterium in IFNAR-deficient cells. It is still unknown whether, in the context of IFNAR-deficient cells, cellular factors alter ActA localization on the bacterial surface or whether this occurs because of altered bacterial regulation. ActA is also critical for avoiding antibacterial autophagy[65,66]. Indeed, in infections with Δ*actA* bacteria, the autophagy adaptor proteins ubiquitin-binding protein p62 (also known as SQSTM1) and nuclear dot protein 52 (NDP52; also known as CALCOCO2) target ubiquitylated bacteria for capture and autophagy by LC3-positive membranes[67]. Furthermore, a double Δ*actA* and Δ*plcA* mutant was even more attenuated than the Δ*actA* mutant, which occurred through increased targeting by autophagy, as PlcA was shown to directly inhibit LC3 modification and decrease phosphatidylinositol 3-phosphate (PI3P) levels[68].

Clathrin
A protein that is important in endocytosis and exocytosis and has heavy chain variants and light chain variants that form a polyhedral lattice on the surface of vesicles.

Sumoylation
The process by which a small ubiquitin-like modifier covalently binds to its substrates. This typically leads to changes in localization or sequestration of transcription factors resulting in transcriptional repression.

Formins
A family of proteins that polymerize actin; each formin can have distinct actin-nucleating properties depending on the family.

Diaphanous formins
A subset of formins that have an autoinhibitory domain that is released by binding to GTPases.

Efferocytosis
The process for phagocytosing dead or dying cells that is initiated by the recognition of phosphatidylserine lipids on the cell surface (lipids normally present on the internal side of the plasma membrane).

Autophagy
A catabolic process that can nonspecifically or selectively capture cytosolic contents, organelles or invading pathogens and target them for degradation in the lysosome.

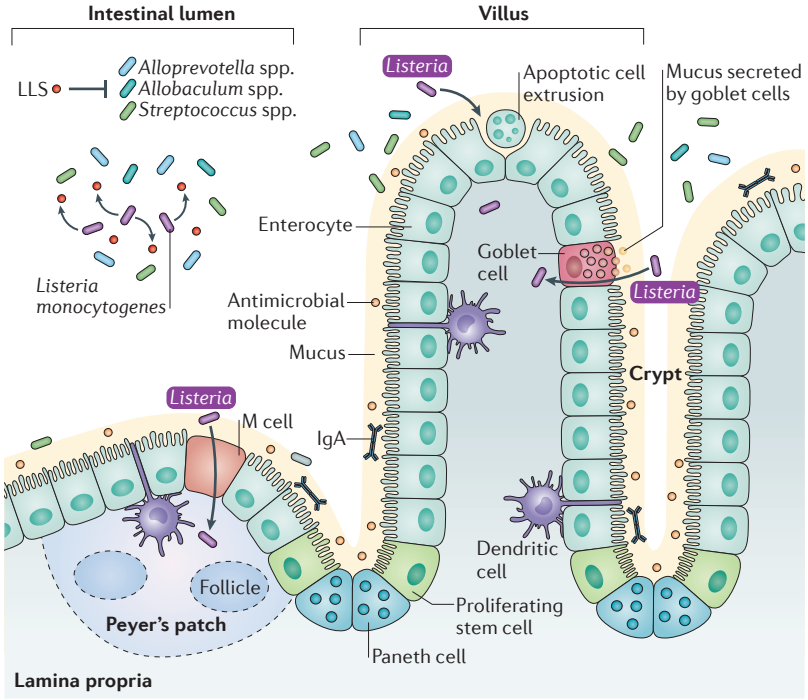


Figure 3 | **Intestinal invasion and interaction of *Listeria monocytogenes* with the microbiota.** In the intestinal lumen, *Listeria monocytogenes* encounters other bacteria that belong to the intestinal microbiota. To restrict the growth of commensal *Alloprevotella*, *Allobaculum* and *Streptococcus* species, *L. monocytogenes* secretes a bacteriocin known as listeriolysin S (LLS) through an as-yet-unknown mechanism. *L. monocytogenes* can enter intestinal villi by accessing surface exposed E-cadherin following apoptotic cell extrusion or at junctions of mucus-secreting goblet cells and enterocytes, through transcytosis of goblet cells. Once the bacterium has invaded the intestinal villi, various cells and effectors contribute to the antibacterial defence system against *L. monocytogenes* infection. For example, Peyer's patches are small masses of lymphatic tissue that are responsible for immune surveillance of the small intestine. Their follicles contain B cells surrounded by T cells. Another line of host defence is represented by dendritic cells that can be found inside Peyer's patches or in the lamina propria of intestinal villi. Some *L. monocytogenes* cells may also be taken up by M cells in the Peyer's patch. This mode of entry occurs at higher frequency for bacteria that express murinized internalin A. In addition, Paneth cells found at the base of the intestinal crypt secrete antimicrobial peptides into the lumen of the intestine. Finally, plasma cells (not shown) secrete immunoglobulin A (IgA). Figure adapted with permission from REF. 129, Elsevier.

Listeria monocytogenes infection *in vivo*

Intestinal, liver and placental invasion. In the intestine, *L. monocytogenes* invades epithelial cells and InlA is the main bacterial player for intestinal infection (FIG. 3). *In vitro*, InlA can interact with human E-cadherin, but not with murine E-cadherin. Therefore, transgenic mice expressing human E-cadherin or knock-in mice expressing humanized E-cadherin were generated[69,70]. InlA mediates bacterial entry into goblet cells and into enterocytes at the tips of intestinal villi, where extrusion of apoptotic intestinal cells occurs. At sites of cell extrusion, multicellular junctions are formed, and InlB, which activates Met, was proposed to cause local increases in receptor recycling and clathrin-mediated endocytosis to increase cytosolic bacterial load in mice[71]. However, *L. monocytogenes* mainly transcytoses across goblet cells that display exposed E-cadherin on their surface[27]. Importantly, pretreatment of mice with interleukin 33 (IL-33) to increase goblet cell abundance dramatically augments *L. monocytogenes* bacterial load in the spleen following oral infection. Moreover, it has been recently shown that goblet cells display constitutively active phosphoinositide 3-kinase (PI3K) signalling[72], explaining why Met activation by InlB at this site of entry is dispensable for transcytosis of *L. monocytogenes*[73]. It is important to note that bacteria that express murinized InlA[74,75] display a different host tropism in the intestine, as entry through Peyer's patches by this variant is mediated by an interaction with N-cadherin rather than with E-cadherin[76]. Moreover, recent work unveiled the relative quantity of intracellular versus extracellular bacteria following food-borne infection in the mesenteric lymph node[77]. Surprisingly, the vast majority of *L. monocytogenes* in this context was found to be extracellular and attached to the surface of a population of activated monocytes[78], although the intracellular population is critical for bacterial spread to the liver and spleen[77]. Of note, following activation, monocytes increasingly express CD64 as they transition into macrophages, and this was found to correlate with bacterial entry, further corroborating the role of CD64 as a receptor for *L. monocytogenes*[78]. In the future, as human organoid cultures and organs 'on a chip' are becoming increasingly experimentally tractable, it will be interesting to compare intestinal invasion of human tissues to murine models of infection.

Following bacterial dissemination to the blood, resident Kupffer cells phagocytose *L. monocytogenes* in the liver. These cells succumb to necroptosis, which generates a specific inflammatory signature that contributes to liver repair through the mobilization of bone-marrow-derived monocytes following infection[79].

Listeriosis is a major health concern during pregnancy. Indeed, a recent prospective study in France highlighted that >83% of maternal listeriosis cases have adverse outcomes for the fetus, ranging from extremely premature birth to fetal death[80]. Diverse animal models as well as *in vitro* systems, such as human placental explants, have indicated an important role for not only InlA but also InlB and ActA in the process of invasion through the placental syncytiotrophoblast layer[11,81–83]. Induction of PI3K signalling by InlB was shown to be critical for the invasion of placental cell lines, murine placental tissue and human explants[72]. Interestingly, extravillous trophoblasts seem to restrict the intracellular growth and spread of *L. monocytogenes,* similarly to phagocytes[84]. In addition, the syncytiotrophoblasts were studied by atomic force microscopy, revealing the unique biophysical properties of this cell layer as a barrier due to its dense meshwork of actin fibres[85]. Furthermore, a recent screen in pregnant guinea pigs has revealed 200 bacterial virulence determinants that are required for placental invasion but do not affect maternal liver colonization[86]. One of these proteins, Lmo2470, is a previously uncharacterized internalin-family protein called InlP[86]. It will be of interest to understand its mechanism of action and cellular target for both *L. monocytogenes* biology and its potential therapeutic relevance during pregnancy.

Necroptosis
A programmed cell death process, distinct from apoptosis, which generates inflammatory signals and typically occurs during infection.

Trophoblasts
Cells that will form the placenta, which are derived from fetal tissue and form the external layer of the developing blastocyst in the context of pregnancy.

Interactions with the intestinal microbiota. Within the intestine, enteropathogens must coexist with the microbiota while evading host antimicrobial defences to gain access to the intestinal epithelium[87]. In order to address the interplay between the microbiota and *L. monocytogenes* infection, a gnotobiotic system was initially used[88]. Germ-free mice were colonized with *Lactobacillus paracasei* or *Lactobacillus casei* before infection with *L. monocytogenes*, and the effect of lactococci colonization on both bacterial and host transcription was monitored[88]. Pre-colonization led to a reduced bacterial burden in a subsequent *L. monocytogenes* infection, with a pronounced effect on immune priming, as the primary colonization induced the production of antibacterial effectors that attenuated the secondary infection. Moreover, infection of animals pre-colonized with lactobacilli stimulated the upregulation of distinct metabolic pathways in *L. monocytogenes*, such as cobalamin biosynthesis and ethanolamine utilization[88]. Pre-colonization also affected host microRNA (miRNA) expression upon infection, particularly of miR-378 and miR-143, whose expression decreased in infection following colonization but was unchanged or higher in infection in germ-free mice[89]. Importantly, a decrease in specific miRNAs was inversely correlated with protein expression of targets that are potentially involved in the immune response to *L. monocytogenes*, such as cyclic AMP-dependent transcription factor (ATF3). Although it is known that germ-free mice are more sensitive to *L. monocytogenes* than conventional mice, a recent groundbreaking study has revealed that the microbiota constitutes a first line of defence against *L. monocytogenes* and established a key role for four Clostridia spp. (*Clostridium saccharogumia*, *Clostridium ramosum*, *Clostridium hathewayi*, now known as *Erysipelatoclostridium ramosum* and *Blautia producta*, now known as *Hungatella hathewayi*)[90]. As these species could potentially be employed as a preventive therapeutic for immunocompromised individuals at high risk of *L. monocytogenes* infection, understanding the mechanism of action used against *L. monocytogenes* and the specificity of these clostridial species will be paramount.

A key factor involved in the interaction of *L. monocytogenes* with the intestinal microbiota was discovered by moving from the analysis of widely used laboratory strains[91] (lineage II strains) to that of clinical isolates of *L. monocytogenes* (lineage I strains; BOX 1). Indeed, a bacterial factor called LLS is absent in laboratory strains but is present in many epidemic strains. LLS is virtually undetectable during growth *in vitro*, although it was initially characterized as a haemolysin important for intraperitoneal infection[92]. Upon closer inspection, in an oral model of listeriosis, this factor was shown to be massively upregulated in the intestine[93]. Strains lacking LLS, compared with wild-type strains, exhibit decreased growth within the intestinal content. LLS was shown to behave like a class of molecules called bacteriocins that target and kill other species of bacteria in the context of interbacterial competition. Forced expression of LLS *in vitro* led to killing of lineage II strains of *L. monocytogenes* that lack this virulence gene, as well as killing of *Lactobacillus delbrueckii* subsp. *lactis* and *Staphylococcus aureus*. Upon infection *in vivo*, LLS specifically targets *Alloprevotella* spp., *Allobaculum* spp. and *Streptococcus spp.* populations, leading to a concomitant bloom in *L. monocytogenes* abundance and virulence (FIG. 3). Both the mechanism of bacterial killing of LLS, whether direct or indirect on *Alloprevotella* spp. and *Allobaculum* spp., and its structure remain to be explored. As *L. monocytogenes* thrives in the soil, a hot spot of interspecies competition, it is likely that LLS could be the first of many bacteriocins expressed by this pathogen. Within the intestinal tract, *L. monocytogenes* must also survive in the presence of potentially antibacterial physiological fluids, such as bile. The master regulator of virulence PrfA and the general stress response regulator RNA polymerase sigma factor (SigB) control the expression of a bile salt hydrolase that mediates catabolism of bile salts[94,95]. In addition, a second protein that was predicted to affect bile tolerance, called BtlB, also has a major influence on *L. monocytogenes* survival in bile and persistence during faecal carriage *in vivo*[95]. An *ex vivo* system using porcine bile to assess survival combined with transposon mutagenesis unearthed that amino acid biosynthesis, purine metabolism and biotin uptake were essential for bacterial survival in these conditions[96].

Bacterial physiology and regulation

Comparative genomics, RNA sequencing technology and proteomics have contributed to a thorough understanding of *L. monocytogenes* regulation. Transcriptomic analyses of wild-type or mutant strains after bacterial growth in various culture conditions, including growth in blood, revealed many small non-coding RNAs[97,98]. Transcription start and termination-site mapping similarly revealed additional information on riboswitches and riboregulators critical to virulence, metabolic processes and antibiotic resistance[99,100]. Finally, unbiased screening approaches have highlighted novel aspects of well-known virulence regulators and have identified unanticipated, potent virulence regulators.

New insights into virulence regulators. PrfA is the major regulator of virulence in *L. monocytogenes* and controls the *L. monocytogenes* pathogenicity island 1 (LIPI-1) from which LLO, PlcA, PlcB, ActA and zinc metalloproteinase (Mpl) are expressed. PrfA belongs to a family of transcription factors that are activated by cofactor binding and has its own complex regulation that acts at transcriptional, translational and protein level (reviewed in REF. 101). Experiments performed over the years have suggested that a factor present in the host cytosol could allosterically activate PrfA; however, until recently, this cofactor has remained elusive. Upon intracellular growth, *actA* is one of the most upregulated PrfA-dependent genes. A forward genetic screen that used an *actA*-deficient strain that possesses the bacterial replication origin flanked by *loxP*

Gnotobiotic
A condition in which the precise contents of the microbiota (bacteria and other microorganisms) of an animal are known; can refer to zero bacteria (germ-free) or a known subset of bacteria.

Immune priming
Transcriptional activation of innate defence pathways or immune memory pathways that leads to a subsequent downstream immune response that is more pronounced than the initial naive immune response.

sites and the *actA* promoter controlling a Cre recombinase from a second plasmid identified glutathione synthase (GshF) as a critical determinant of PrfA activation. This was an indication that glutathione, a molecule that is present in bacteria and mammalian cells, could be the potential PrfA cofactor. The Δ*gshF* mutant displayed reduced ActA expression and intracellular growth, and this phenotype was rescued by complementation with a constitutively active mutant of PrfA, called PrfA*(REF. 102). Interestingly, PrfA affinity for the promoter regions of *hly,* the gene that encodes LLO, and *actA* was dependent on oxidation levels of glutathione. The structural basis of PrfA in complex with glutathione and DNA was subsequently solved, further supporting these findings[103]. Although PrfA controls LIPI-1, a two-component system virulence regulator (VirR–VirS), identified by signature tagged mutagenesis, regulates virulence through the expression of 17 genes, several of which affect bacterial cell wall and membrane integrity[104,105]. Deletion of *virR* sensitizes bacteria to antimicrobial compounds through the effect of this regulator on D-alanylation of lipoteichoic acids mediated by the *dltABCD* locus and lysinylation of phospholipids mediated by the multiple peptide resistance factor (MprF)[104,106,107]. Future work on protein and non-coding RNA regulation controlled by VirR will be of interest to further elucidate its role in antimicrobial resistance.

Small non-coding RNAs. Small non-coding RNAs can add a layer of fine-tuning to gene expression, which allows the pathogen to efficiently alternate between distinct environments. Transcriptomic analysis and single base pair resolution mapping of the transcription start site after bacterial growth in various conditions identified at least 154 sense RNAs and 86 antisense RNAs[97–99,108], many of which have yet to be characterized. Rli31 was identified in a screen for lysozyme sensitivity as the only small RNA required in addition to 12 protein-coding genes[109]. The peptidoglycan of the *rli31* deletion strain had fewer *N*-deacetylated glycans and altered crosslinking, which correlated with aberrant regulation of two other targets of the screen, *pgdA* (peptidoglycan *N*-deacetylase) and *pbpX* (putative penicillin-binding protein PbpX), a peptidoglycan deacetylase and a putative carboxypeptidase, respectively[110]. Mutants were rapidly cleared from the blood; however, the mechanism of this regulation is enigmatic, as Rli31 does not have complementarity for the transcripts of these target proteins. A suppressor screen identified mutations in the promoter region of the *spoVG* operon, which plays very diverse roles in different bacteria from antibiotic resistance in *S. aureus* to growth and sporulation in *Bacillus subtilis*, exhibits nearly perfect complementarity with Rli31 and seems to bind to most if not all other small RNAs that were tested. Although these mutations did not affect mRNA or protein levels of SpoVG, *spoVG* deletion had pleiotropic and unrelated effects on motility, lysozyme resistance and carbon metabolism. Thus, the authors hypothesized that SpoVG acts as a global post-transcriptional regulator through its capacity to bind non-coding RNA[111]. A second *L. monocytogenes* small RNA, Rli27, has recently been characterized and was shown to be implicated in the regulation of surface expression of Lmo0514 protein, an LPXTG-motif protein that is important for virulence[112]. In this case, Lmo0514 is transcribed from two distinct promoters that generate two transcripts that differ in the length of their 5′ UTRs (FIG. 4a). The long transcript of Lmo0514 and Rli27 are both upregulated during intracellular growth. Rli27 acts in *trans* and binds to the 5′ UTR of the long form of Lmo0514. Rli27 binding alters the transcript structure to reveal a ribosome-binding site that leads to increased protein translation. Ultimately, two modes of regulation at the transcriptional and translational level ensure that Lmo0514 is produced intracellularly. Taken together, both examples (Rli31 and Rli27) highlight the importance of small RNA-mediated regulation for bacterial fitness during environmental or intracellular growth.

Riboswitches and riboregulators. One striking aspect of *L. monocytogenes* physiology is the abundance of riboswitches and riboregulators that can sense small molecules and adjust biosynthetic or antibiotic resistance pathways accordingly. A riboswitch is an RNA element upstream of a coding gene that can have two alternative structures. Binding of small molecules and cofactors to the riboswitch results in a change in secondary structure that generally leads to the production of a shorter transcript. Riboswitches are typically present upstream of protein-coding genes or operons. Recently, a non-canonical riboswitch that controls a locus required for propanediol catabolism has been identified[113] (FIG. 4b). This vitamin B_{12} riboswitch controls an antisense RNA called AspocR (previously thought to be two distinct small RNAs, Rli39 and RliH) that inhibits PocR expression when expressed as a long transcript in the absence of vitamin B_{12}. PocR is a transcription factor that controls the *pdu* genes, which mediate propanediol usage, whereas vitamin B_{12} is an essential cofactor for the Pdu proteins, which are thus expressed only in the presence of vitamin B_{12} (FIG. 4b). Indeed, in the presence of vitamin B_{12}, only the short transcript of AspocR is expressed and does not control PocR.

Analysis of a second *L. monocytogenes* vitamin B_{12} riboswitch revealed an entirely novel mode of regulation, which combines a riboswitch regulating a non-coding RNA with two-component signalling (FIG. 4c). The vitamin B_{12} riboswitch controls a non-coding RNA, Rli55, which can be expressed either as a short or as a long transcript. The long transcript contains an ANTAR element that can sequester a regulatory protein, named EutV, which is part of a two-component signalling system, whereas the short transcript cannot bind it[114]. In addition, the two-component system is unusual as it is composed of the kinase EutW and EutV, which acts as an anti-terminator. EutW senses ethanolamine levels leading to upregulation of the *eut* operon to regulate ethanolamine utilization enzymes,

ANTAR element
An RNA-binding domain called AmiR and NasR transcriptional anti-terminator regulator (ANTAR).

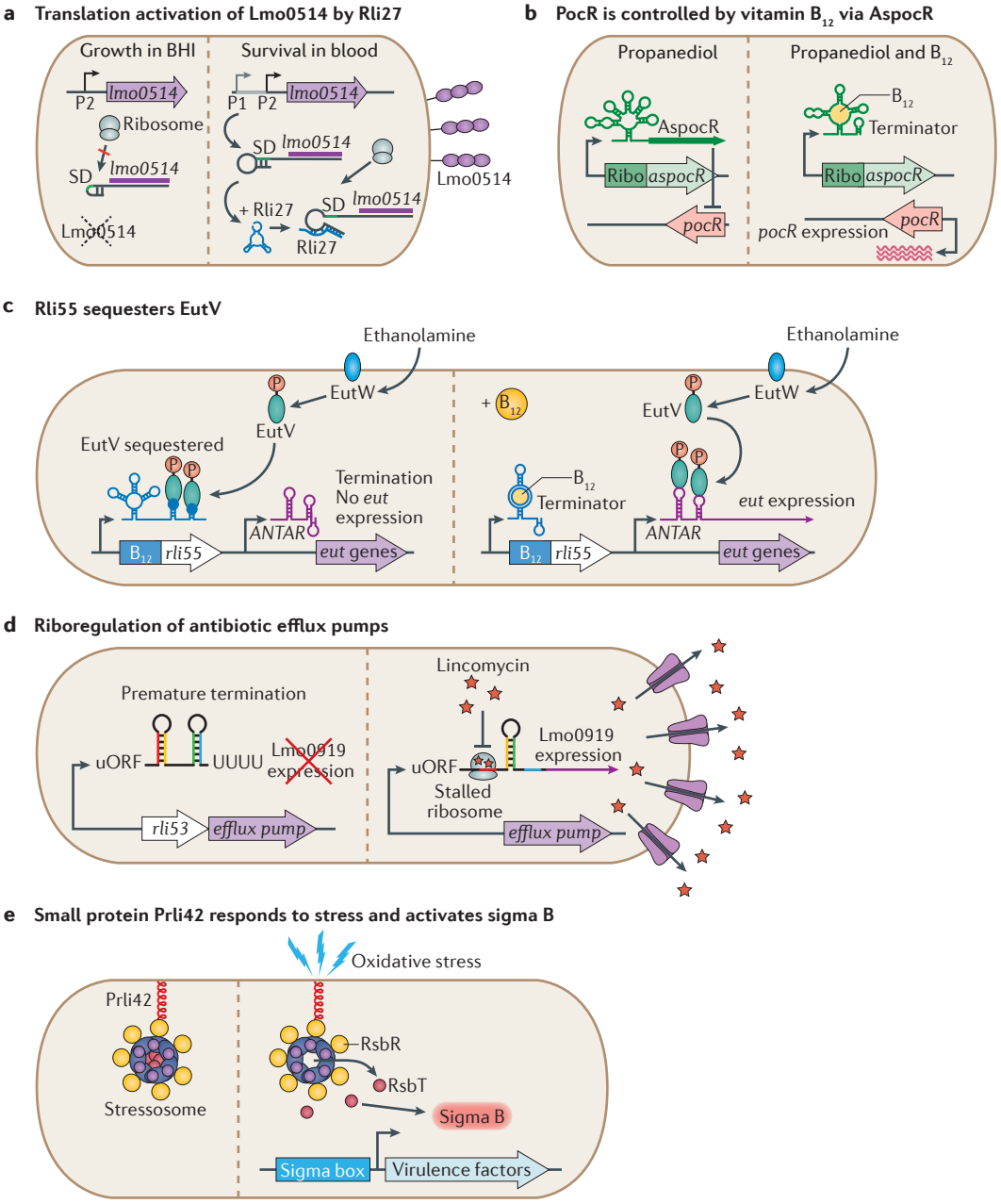


Figure 4 | **RNA regulation and miniproteins in *Listeria monocytogenes*. a** | Expression of Lmo0514 protein is controlled both transcriptionally and translationally. *lmo0514* can be transcribed in a long form or in a short form. The long form is upregulated in blood and binds to the small non-coding RNA Rli27, exposing a ribosome-binding site that leads to increased translation of Lmo0514. As Rli27 is not upregulated in brain–heart infusion (BHI) medium, only the short form is expressed under these conditions. **b** | Expression of regulatory protein PocR is controlled by vitamin B_{12} binding to a long antisense RNA called AspocR. In the absence of vitamin B_{12}, AspocR reduces PocR expression, whereas in the presence of vitamin B_{12}, premature termination of AspocR leads to upregulation of PocR. **c** | Rli55 sequesters EutV, which acts as an anti-terminator and is part of a two-component system for ethanolamine utilization, through a binding domain on Rli55 called an AmiR and NasR transcriptional anti-terminator regulator (ANTAR) element. In the absence of vitamin B_{12}, the riboswitch Rli55 binds to EutV. If vitamin B_{12} is present, Rli55 is truncated into a short form that cannot bind EutV, allowing EutV to derepress *eut* gene expression. **d** | The riboregulator Rli53 controls lincomycin resistance mediated by Lmo0919 protein. In the absence of the antibiotic lincomycin, Rli53 is in a closed conformation, resulting in transcriptional termination before *lmo0919*. Following antibiotic-induced ribosomal stalling, Rli53 secondary structure changes, leading to overexpression of Lmo0919 and lincomycin efflux. **e** | The small membrane protein Prli42 anchors the stressosome to the bacterial membrane and controls the Sigma B-dependent oxidative stress response that is activated following hydrogen peroxide treatment or growth in macrophages. The stressosome sequesters the serine/threonine-protein kinase RsbT, which is released following stress and can indirectly activate RNA polymerase sigma factor SigmaB through a phosphorylation–dephosphorylation cascade (not shown). P, phosphate; P1, Promoter 1; P2, Promoter 2; SD, Shine–Dalgarno; U, uracil; uORF, upstream ORF. Parts **a**, **b** and **c** are adapted with permission from REF. 130, Annual Reviews.

Attenuation-like mechanism
A mechanism of transcriptional control in bacteria and archaea that incorporates a terminator sequence into the 5′ mRNA leader that can stall the ribosome (resulting in aborted translation) or allow readthrough depending on metabolic conditions.

Ribosomal stalling
An event that occurs when the ribosome slows during translation, often owing to a specific secondary structure in the mRNA, resulting in aborted translation or temporary ribosomal pausing.

which are important for bacterial metabolism in the microbiota. The anti-terminator EutV functions by directly binding to a distinct ANTAR element in the 5′UTR of the *eut* genes, thus allowing their transcription. In the absence of vitamin B_{12}, Rli55 acts as a sink to sequester EutV, which can then no longer bind to the 5′UTR of *eut* genes. As ethanolamine utilization enzymes also require vitamin B_{12} as a cofactor, this mode of regulation integrates several tiers of sophisticated regulation for survival in the intestine, in which ethanolamine is abundant and generated by commensals in the microbiota.

Riboregulators also play a role in the increasingly critical health problem of antibiotic resistance. By use of an innovative approach called 3′ term-seq to identify transcriptional termination sites in an unbiased fashion, new riboregulators were identified in *Bacillus* spp., *L. monocytogenes* and *Enterococcus faecalis*[100]. This method was used to assess transcription after treatment with metabolites, antibiotics and growth in monobacterial cultures or in the oral microbiota. Strikingly, a series of terminators and anti-terminators were found upstream of efflux pump genes[100]. In *L. monocytogenes*, a new lincomycin resistance gene, called *lmo0919*, encoding an ABC transporter, was identified[100] (FIG. 4d). This gene is regulated by an attenuation-like mechanism that generates a small transcript, which encodes a micro-ORF and was previously identified as Rli53. In the absence of lincomycin, the riboregulator leads to premature termination of the transcript. Upon antibiotic treatment, ribosomal stalling on the ORF leads to a longer transcript that allows expression of *lmo0919* and increases efflux of cytoplasmic antibiotics. Moreover, this study has shown that riboregulators appear to be much more numerous than previously anticipated.

Bacterial stress responses: miniproteins and kinases. Cell wall integrity is critical for stress responses, antibiotic resistance, intracellular growth and virulence, as evidenced by the dramatic attenuation of virulence in mutants that lack the peptidoglycan modification enzymes PgdA[110], *O*-acetyltransferase (OatA)[109,115], D-alanine-poly(phosphoribitol) ligase subunit 1 (DltA) and the membrane modifier phosphatidylglycerol lysyltransferase (MprF). The penicillin-binding-protein and serine/threonine associated kinase PrkA is conserved in *B. subtilis* and *Mycobacterium tuberculosis*; however, its function in *L. monocytogenes* physiology had not been previously assessed. PrkA is critical for *L. monocytogenes* cell wall stress responses, intracellular survival and virulence *in vivo*. PrkA phosphorylates YvcK, whose function is unknown but whose deficiency phenocopies that of deficiency of PrkA[116]. As bacteria deficient in both PrkA and YvcK are substantially attenuated *in vivo*, it will be of interest to further elucidate the role of both virulence factors in mechanistic detail.

L. monocytogenes has different modes of activation of stress response genes. The stressosome is a supramolecular stress-sensing complex, which in *B. subtilis* sequesters an effector kinase that is released upon various bacterial stress conditions, thereby activating SigB-dependent stress response genes[117]. By using an N-terminomics approach to identify translation initiation sites in *L. monocytogenes*, a number of novel internal translation initiation sites and six miniproteins were discovered[118], including Prli42. Prli42 is highly conserved in Firmicutes; it is localized in the membrane and anchors the stressosome there (FIG. 4e). Deletion of the peptide sensitizes bacteria to oxidative stress and macrophage killing. Taken together, these findings suggested that Prli42 could bring the sensory protrusions of the stressosome to the membrane to locally sense stress on the bacterial surface, where it could activate the stressosome through a conformational change or bring the stressosome into contact with an as-yet-unidentified membrane protein. Future structural work should help distinguish among these hypotheses to determine the precise mode of action of Prli42.

Conclusions and future directions

L. monocytogenes is a human pathogen whose varied and complex mechanisms of regulation and diverse responses to stress allow it to survive in highly distinct environmental conditions and to switch from saprophytism to virulence. In the past ten years genomics, transcriptomics, 5′ and 3′ RNA sequencing, proteomics and forward genetic screens have led to a plethora of new information on the mechanisms of action of both this bacterium and the host. In the future, the molecular basis of differences between clinical and environmental strains may reveal new virulence factors and pathogenic mechanisms. By investigating the epigenetic response to infection, novel roles of histone writers and erasers have been revealed, and further investigation into the epigenetic memory of infection will undoubtedly be important, particularly in long-lived cells. Genome-wide gain-of-function and loss-of-function screens have linked largely unstudied host proteins to bacterial infection, and exploring their function will be critical for both eukaryotic cell biology and infection biology. Innate immune signalling paradigms have been explored and expanded thanks to the abundance of secreted bacterial factors that bind to host sensors, and understanding the role of these pathways in novel tissue models will be informative for human listeriosis. In addition, unanswered questions remain regarding infection processes *in vivo*; in particular, mechanisms for crossing of the fetoplacental barrier and blood–brain barrier as well as the long period of latency in human infection, which does not occur during murine infection, remain to be resolved. The discovery of a *L. monocytogenes* bacteriocin and its effect on the microbiome has raised questions about how the bacterium interacts with diverse bacterial communities both in the human microbiome and in the soil. A new era in microbiology research has begun, which in the coming years should reveal answers to many critical questions about how *L. monocytogenes* establishes and maintains a productive infection in human hosts and how its specific infection strategies and attributes compare to other enteropathogens.

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