CHAPTER ONE

New Insights into Determinants of *Listeria Monocytogenes* Virulence

Olivier Dussurget[*,†,‡]

Contents

* Institut Pasteur, Unité des Interactions Bactéries-Cellules, Paris F-75015, France
† Inserm, U604, Paris F-75015, France
‡ INRA, USC2020, Paris F-75015, France

International Review of Cell and Molecular Biology, Volume 270
ISSN 1937-6448, DOI: 10.1016/S1937-6448(08)01401-9

Abstract

Listeria monocytogenes is the causative agent of human listeriosis, a potentially fatal foodborne infection. Clinical manifestations range from febrile gastroenteritis to more severe invasive forms including meningitis, encephalitis, abortions, and perinatal infections. This Gram-positive facultative intracellular pathogen has evolved multiple strategies to face extracellular innate defense mechanisms of the host and to invade and multiply intracellularly within macrophages and nonphagocytic cells. This chapter provides an updated panorama of recent advances in the characterization of *L. monocytogenes* virulence determinants in the postgenomic era.

Key Words: Listeriosis, *Listeria monocytogenes*, Virulence, Genome, Cell invasion, Immunity, Pathophysiology.

1. Introduction

The *Listeria* genus is composed of six species: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, and *L. grayi* (Vazquez-Boland *et al.*, 2001b). The pathogenic species *L.monocytogenes* causes disease in humans and animals. The second pathogenic species, *L. ivanovii*, causes disease in animals. *L. innocua*, *L. seeligeri*, *L. welshimeri*, and *L. grayi* are four nonpathogenic species. *Listeria* spp. are flagellated and motile Gram-positive, nonspore-forming, facultative anaerobic bacilli of low GC content (Seeliger and Jones, 1986). These ubiquitous bacteria are commonly isolated from multiple sources such as plants, soil, and water. *L. monocytogenes* can contaminate the agricultural environment, animal feed, and food at various stages of the production process leading to recalls (Orndorff *et al.*, 2006; Roberts and Wiedmann, 2003). It is thus a major problem in the food industry. Ingestion of food contaminated with *L. monocytogenes* is the primary route of transmission to humans. *L. monocytogenes* is the causative agent of listeriosis. Although the incidence of the disease is low (0.1 to 11.3/1,000,000), it is a public health concern because of a high mortality rate (20–30%) and high occurrence of *Listeria* in food (Swaminathan and Gerner-Smidt, 2007).

L. monocytogenes causes two forms of listeriosis depending on the immunological status of the host, the pathogenic potential of the bacterial strain, and the infectious dose: noninvasive gastrointestinal listeriosis and invasive listeriosis (Vazquez-Boland *et al.*, 2001b). In immunocompetent individuals, noninvasive listeriosis develops as a typical febrile gastroenteritis. In immunocompromised adults such as the elderly, patients with genetic or acquired defects in immunity and patients receiving immunosuppressive agents, listeriosis can manifest as septicemia and/or meningoencephalitis.

Invasive listeriosis can also be acquired by the fetus from the infected mother by transplacental transmission. Perinatal listeriosis can lead to abortion, birth of a stillborn fetus or a baby with generalized infection (granulomatosis infanseptica), and meningitis in neonates. Clinical features of invasive listeriosis derive from the unique capacity of *L. monocytogenes* to cross three barriers: the intestinal, blood–brain, and placental barriers (Lecuit, 2005). The clinical outcome of listeriosis is influenced by the pathogenic potential of the infecting strain. Among *L. monocytogenes* strains, those of the serovars 1/2a, 1/2b, and 4b are responsible for 95% of human infections and most outbreaks are caused by strains of serovar 4b (Swaminathan and Gerner-Smidt, 2007). The remarkable capacity of *L. monocytogenes* to invade and multiply in epithelial cells and professional phagocytic cells is central to listeriosis pathophysiology (Fig. 1.1). *L. monocytogenes* uses various receptors to enter these cells. After internalization, the bacterium lyzes the vacuole, escapes in the cytosol, and replicates. *L. monocytogenes* then exploits the actin machinery to move within the cell and to neighboring cells where it is internalized in a double-membrane vacuole that is lyzed, allowing the bacterium to access the cytosol and start a new intracellular infection cycle (Tilney and Portnoy, 1989).

For more than 40 years, *L. monocytogenes* and experimental listeriosis have been used to study the immune response and the biology of the cell leading to major discoveries (Cossart, 2007; Garifulin and Boyartchuk, 2005; Hamon *et al.*, 2006; Mackaness, 1962; Pamer, 2004). More recently, the extensive characterization of the mechanisms used by *L. monocytogenes* to

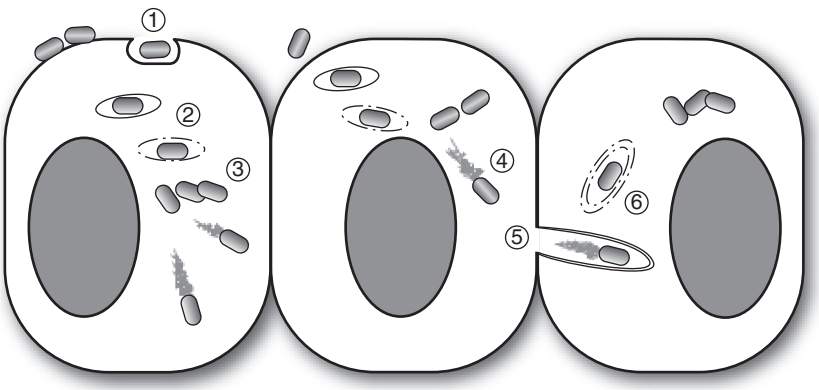

Figure 1.1 Schematic representation of the infectious cycle of *Listeria monocytogenes*. Bacteria first adhere to cells, induce entry, and are internalized in a vacuole (1). The vacuole is lyzed by *L. monocytogenes* virulence factors (2). Once free in the cell cytoplasm, bacteria start to replicate (3). *L. monocytogenes* then exploits the actin polymerization machinery of the cell to propel itself (4). When bacteria reach a neighboring cell, they induce the formation of a protrusion characterized by a double membrane (5). This secondary vacuole is finally lyzed (6), allowing a new infection cycle.

manipulate the host cell contributed to the creation of the field of cellular microbiology (Cossart *et al.*, 1996). The advanced knowledge of the specificity of *L. monocytogenes* interactions with the host culminated in 2001, with the creation of the first transgenic mouse to model human listeriosis in animals (Lecuit, 2007; Lecuit and Cossart, 2002; Lecuit *et al.*, 2001). The same year, the first comparison of the genome sequences of a pathogenic bacterium and a related nonpathogenic species, *L. monocytogenes* and *L. innocua*, respectively, allowed to envision the identification of the complete arsenal used by *Listeria* to cause disease (Dussurget *et al.*, 2004; Glaser *et al.*, 2001). Here, we review *L. monocytogenes* major virulence determinants that have been currently characterized.

2. Acquisition of Virulence Genes and Their Expression

2.1. Acquisition of virulence genes

Acquisition and loss of genetic elements lead to bacterial speciation and provide the properties necessary for a particular lifestyle. Cumulative acquisition of virulence genes provides pathogenic bacteria the functions required for survival, growth and damage in the infected host.

The genome organization is remarkably conserved between different *Listeria* species (Hain *et al.*, 2007; Schmid *et al.*, 2005). However, comparative genomic analysis of pathogenic and nonpathogenic *Listeria* species reveals scattered genes specific to virulent strains that are isolated or form pathogenicity islands (Glaser *et al.*, 2001; Vazquez-Boland *et al.*, 2001a). The unusual base composition of some of these sequences could be the consequence of horizontal transfer (Begley *et al.*, 2005; Dussurget *et al.*, 2002). Interestingly, *Listeria* genomes contain open reading frames homologous to *Bacillus subtilis* competence genes (Buchrieser, 2007; Glaser *et al.*, 2001). Although the function of this putative DNA uptake system has not been demonstrated yet, it could be hypothesized that competence may play a role in acquisition of virulence genes by *L. monocytogenes*. Lysogenic bacteriophages, plasmids, and transposons, which could play critical roles in the evolution of pathogenicity, have been characterized in *Listeria* species but they have not been associated to virulence (Hain *et al.*, 2007). Nonpathogenic species, including *L. innocua* appear to have evolved from a *L. monocytogenes* ancestor after multiple deletions of virulence genes (Buchrieser, 2007; Hain *et al.*, 2006). Recently, analysis of the complete genome sequence of the nonpathogenic *L. welshimeri* revealed deletions of all the genes required for virulence and of some genes encoding

transcription factors, surface proteins, and proteins involved in carbohydrate transport and metabolism (Hain *et al.*, 2006). Comparison of *L. welshimeri* and *L. innocua* suggests similar evolutionary paths from an ancestor.

2.2. Regulation of virulence gene expression

Adaptability of *L. monocytogenes* that is central to the infectious process is determined by the genetic elements allowing bacteria to survive and multiply within multiple tissues and by the mechanisms required for the tight and coordinate regulation of their expression.

2.2.1. PrfA

PrfA is the master regulator of virulence gene expression in *L. monocytogenes*. PrfA is a protein of 233 amino acids that binds to a 14-bp palindromic sequence in the −41 region of the genes from the PrfA regulon and activates their transcription. The activity of PrfA itself is tightly controlled by multiple mechanisms (Vazquez-Boland *et al.*, 2001b). Translation of PrfA is regulated by temperature (Johansson and Cossart, 2003; Johansson *et al.*, 2002). At a temperature lower than 30 °C, the untranslated region of *prfA* mRNA adopts a stable secondary structure that prevents binding of the ribosome and blocks translation. In the host, the temperature of 37 °C induces melting of the secondary structure. Consequently, PrfA is translated and activates virulence gene expression. Determination of *L. monocytogenes* genome sequence allowed analysis of the transcriptome and identification of the PrfA regulon (Milohanic *et al.*, 2003). The transcriptomic analysis identified a total of 73 genes regulated directly or indirectly by PrfA. This study confirmed that the expression of important virulence genes such as *hly*, *actA*, *plcA*, *plcB*, *mpl*, *inlA*, *inlB*, *inlC*, *hpt*, and *prfA* itself is activated by PrfA. Interestingly, the expression of all these genes is increased intracellularly after infection of macrophages and epithelial cells (Chatterjee *et al.*, 2006; Joseph *et al.*, 2006).

2.2.2. Sigma B

Other regulatory elements have been demonstrated to be necessary for full virulence of *L. monocytogenes*. The stress-responsive alternative sigma factor encoded by *sigB* contributes to invasion (Kim *et al.*, 2004) and virulence (Garner *et al.*, 2006; Nadon *et al.*, 2002). The sigma B regulon contains stress response and virulence genes such as *gadB*, *opuCA*, *bsh*, *inlA*, and *inlB* (Kazmierczak *et al.*, 2003; McGann *et al.*, 2007; Sue *et al.*, 2003) and stress and virulence gene regulators Hfq (Christiansen *et al.*, 2004; Nadon *et al.*, 2002) and PrfA (Nadon *et al.*, 2002).

2.2.3. MogR

Temperature-dependent expression of the flagellin gene *flaA* is controlled by the transcriptional regulator DegU and by the antagonist activity of the repressor MogR (Grundling *et al.*, 2004). At 37 °C, flagellin synthesis is repressed by the regulator MogR. At 30 °C and below, DegU activates expression of GmaR that forms a complex with MogR and prevents binding of the repressor to its target DNA sequences (Shen *et al.*, 2006). GmaR is a bifunctional protein that functions as an antirepressor and an O-linked *N*-acetylglucosamine transferase that glycosylates flagellin (Schirm *et al.*, 2005; Shen *et al.*, 2006). The role of flagellin glycosylation remains to be determined. MogR contributes to *L. monocytogenes* virulence (Grundling *et al.*, 2004; Shen and Higgins, 2006) and its expression is induced in macrophages (Chatterjee *et al.*, 2006). Overproduction of FlaA in *mogR* mutants leads to defects in bacterial division, intracellular spread, and virulence in mice.

2.2.4. CtsR

The class III stress gene repressor CtsR regulates the expression of class III heat-shock genes encoding the Clp ATPases ClpB, ClpC, ClpE, and ClpP, which are required for virulence (Chastanet *et al.*, 2004; Gaillot *et al.*, 2000; Nair *et al.*, 1999, 2000; Rouquette *et al.*, 1998). Interestingly, the expression of CtsR and the four ATPases is induced in infected macrophages (Chatterjee *et al.*, 2006).

2.2.5. PerR and Fur

The Fur family of regulators includes sensors of iron (Fur), zinc (Zur), manganese (Mur), nickel (Nur), as well as metal-dependent reactive oxygen species sensors such as the peroxide sensor PerR (Lee and Helmann, 2007). The iron-responsive transcriptional regulator Fur is responsible for coordinating the expression of genes involved in iron uptake and storage (Lee and Helmann, 2007). The regulator PerR senses peroxides by metal-catalyzed oxidation and regulates the expression of inducible genes involved in defense against reactive oxygen species (Lee and Helmann, 2006). *L. monocytogenes perR* and *fur* mutants are more sensitive to hydrogen peroxide and have a significantly reduced virulence of in mice (Rea *et al.*, 2004, 2005). Interestingly, the PerR regulon includes the ferritin gene *fri* that contributes to survival of *L. monocytogenes in vivo* (Dussurget *et al.*, 2005; Mohamed *et al.*, 2006; Olsen *et al.*, 2005). Thus, regulation of iron uptake and oxidative stress response is an important determinant for the infectious process.

2.2.6. LisRK, AgrA, VirR, and DegU

Several two-component regulatory systems contribute to *L. monocytogenes* survival in the infected host. LisRK is important for bacterial response to acid and hydrogen peroxide stresses and for osmotolerance mediated by the

HtrA-like serine protease (Cotter *et al.*, 1999; Stack *et al.*, 2005). The response regulators AgrA (Autret *et al.*, 2003) and VirR (Mandin *et al.*, 2005) play a role in virulence, which was identified by signature-tagged mutagenesis. A transcriptomic approach led to the identification of 12 genes regulated by VirR, including the *dlt* operon, which is required for *L. monocytogenes* full virulence. However, a *dltA* mutant is not as impaired in virulence as a *virR* mutant, suggesting that the response regulator may control the expression of other virulence determinants (Mandin *et al.*, 2005). Indeed, another member of the VirR regulon, the *mprF* gene, has recently been shown to contribute to *L. monocytogenes* virulence (Thedieck *et al.*, 2006). The response regulator DegU is a transcriptional activator of the expression of the flagellin gene *flaA* at low temperature and regulates virulence-associated genes (Knudsen *et al.*, 2004; Williams *et al.*, 2005).

2.2.7. Stp

Analysis of *L. monocytogenes* genome sequence revealed 9 signal transduction systems based on reversible phosphorylation in addition to the 16 two-component systems: 4 putative tyrosine phosphatases, 3 putative serine-threonine kinases, and 2 putative serine-threonine phosphatases (Archambaud *et al.*, 2005; Glaser *et al.*, 2001). One of the latter enzyme is an Mn^{2+}-dependent serine-threonine phosphatase that has an important role in regulating the elongation factor EF-Tu and controlling bacterial survival in the infected host (Archambaud *et al.*, 2005). Stp was recently shown to control L. monocytogenes manganese dependent-superoxide dismutase (MnSOD) an enzyme that is required for full virulence (Archambaud *et al.*, 2006).

2.2.8. Hfq

The RNA-binding protein Hfq regulates multiple important processes such as stress tolerance and virulence. Hfq contributes to virulence in mice possibly by interacting with mRNA and/or small regulatory RNA, playing a role in the survival and multiplication of *L. monocytogenes in vivo* (Christiansen *et al.*, 2004; Mandin *et al.*, 2007).

3. Adaptation to Host Extracellular Compartments

Following ingestion, the capacity of *L. monocytogenes* to survive and multiply successfully under the multiple and dynamic environments found in the host is an essential factor in the infectious process.

3.1. GAD

The glutamate decarboxylase system GAD is essential for survival in the stomach after ingestion (Cotter *et al.*, 2001). Depending on the strain, it is composed of two or three glutamate decarboxylases and one or two glutamate/γ-aminobutyrate antiporters (Cotter *et al.*, 2005). The GAD system transports and converts glutamate to γ-aminobutyrate consuming a proton, allowing *L. monocytogenes* to survive in acidic environments.

3.2. BSH

Bile is essential to emulsify lipids and has important antimicrobial properties. *L. monocytogenes* is well equipped to tolerate high concentration of bile (Begley *et al.*, 2002, 2003, 2005; Dussurget *et al.*, 2002; Sleator *et al.*, 2005). Analysis of *L. monocytogenes* genome sequence revealed the presence of a gene encoding a bile salt hydrolase (BSH) that was absent from the genome of the nonpathogenic species *L. innocua* (Dussurget *et al.*, 2002). BSH is produced by commensal enteric bacteria and lactic bacteria. Deconjugation of conjugated bile salts by BSH could be a protective mechanism against bile toxicity. *L. monocytogenes* BSH is controlled by sigma B (Kazmierczak *et al.*, 2003; Sue *et al.*, 2003) and activated by PrfA (Dussurget *et al.*, 2002). Its activity is induced at low oxygen tension that could be a signal sensed by bacteria after ingestion to express the *bsh* as well as other virulence genes (Dussurget *et al.*, 2002). *L. monocytogenes* BSH confers resistance to bile (Begley *et al.*, 2005; Dussurget *et al.*, 2002). Deletion of the *bsh* gene results in dramatically reduced fecal carriage in guinea pigs after intragastric inoculation and decreased survival in the liver of mice after intravenous injection (Dussurget *et al.*, 2002). BSH is therefore a new type of virulence determinant that is important for both intestinal persistence and hepatic colonization.

3.3. BilE

Analysis of *L. monocytogenes* genome revealed a two-gene operon, *bilEA–bilEB*, which is critical for bile tolerance (Sleator *et al.*, 2005). The expression of the operon is controlled by sigma B and PrfA. The operon encodes a bile exclusion system providing a protection against bile and contributing to *L. monocytogenes* virulence in mice infected orally.

3.4. BtlB

A third locus, *btlB*, has been shown to contribute to bile tolerance and *L. monocytogenes* virulence in mice (Begley *et al.*, 2005). BtlA and Pva that encode a putative transporter and a penicillin V amidase, respectively, are other important determinants conferring tolerance to bile but do not contribute significantly to virulence in mice (Begley *et al.*, 2003, 2005).

3.5. OpuC

Once in the intestinal lumen, *L. monocytogenes* has to cope not only with the presence of bile salts but also with an increased osmolarity. *L. monocytogenes* produces several osmolyte uptake systems increasing osmotolerance, such as the glycine betaine transporters BetL and Gbu and the carnitine transporter OpuC (Ko and Smith, 1999; Sleator *et al.*, 1999, 2001; Wemekamp-Kamphuis *et al.*, 2002). While deletion of *betL* and *gbu* does not affect virulence, OpuC is required for full virulence in mice infected orally (Sleator *et al.*, 2001; Wemekamp-Kamphuis *et al.*, 2002).

3.6. OppA

Uptake of oligopeptides by the OppA transporter could also contribute to osmotolerance and is required for intracellular survival in macrophages and bacterial growth in mice (Borezee *et al.*, 2000).

4. Adhesion, Cell Invasion, and Intracellular Multiplication

Following gastrointestinal passage of *L. monocytogenes*, some of the bacteria that survived nonspecific defense mechanisms of the host in the stomach and intestinal lumen invade the intestinal tissue. Crossing of the intestinal barrier prevents their mechanical elimination by peristalsis and competition with the commensal flora. *L. monocytogenes* has the capacity to invade both intestinal epithelial cells and M cells of Peyer's patches. After intestinal translocation, bacteria reach the liver, spleen, and mesenteric lymph nodes by the blood and lymph. In the liver, the major site of *L. monocytogenes* multiplication is the hepatocyte. If the multiplication is not controlled by the host immune response, bacteria access the blood-stream and infect secondary target organs. Although *L. monocytogenes* has a strong neurotropism, it can infect a wide range of tissues (Vazquez-Boland *et al.*, 2001b). *L. monocytogenes* has an exceptional repertoire of virulence determinants involved in cellular adhesion, entry, and survival (Bierne and Cossart, 2007; Hamon *et al.*, 2006; Seveau *et al.*, 2007).

4.1. Adhesion

4.1.1. Ami

Ami is a 102-kDa autolytic amidase of 917 amino acids that is involved in adhesion to cells and virulence (Milohanic *et al.*, 2000, 2001). It was identified by transposon mutagenesis in an *inlAB* deletion mutant

(Milohanic *et al.*, 2000, 2001). One of the mutants severely defective in adhesion to eukaryotic cells had five insertions, one of which was upstream from the *ami* gene. Construction of an *ami* null mutant demonstrated that Ami significantly contributed to *L. monocytogenes* adhesion capacity (Milohanic *et al.*, 2000, 2001). Ami has an N-terminal region containing the amidase domain and C-terminal cell wall-anchoring domain composed of eight modules containing the dipeptide GW (Milohanic *et al.*, 2004). Adhesion to cells is promoted by the cell wall-anchoring domain (Milohanic *et al.*, 2000, 2001). *L. monocytogenes* attachment mediated by Ami may contribute to colonization of host tissues.

4.1.2. DltA

Lipoteichoic acids are highly anionic cell wall-associated polymers. The *dltABCD* operon is responsible for D-alanine esterification of lipoteichoic acids. Inactivation of the D-alanine-polyphosphoribitol ligase gene *dltA*, leading to synthesis of D-alanine-deficient lipoteichoic acids, attenuates *L. monocytogenes* virulence in mice (Abachin *et al.*, 2002; Mandin *et al.*, 2005). DltA deficiency decreases adherence of bacteria to macrophages, hepatocytes, and epithelial cells, possibly by modulation of the charge of the bacterial surface and/or by alteration of adhesin-binding activity (Abachin *et al.*, 2002).

4.1.3. FbpA

FbpA is an adhesin that is important for *L. monocytogenes* pathogenesis. FbpA has been identified using signature-tagged mutagenesis (Dramsi *et al.*, 2004). It was shown to be required for liver colonization of mice inoculated intravenously as well as intestinal and liver colonization of mice expressing human E-cadherin after intragastric inoculation. FbpA is a protein of 570 amino acids homologous to atypical fibronectin-binding proteins. It binds to human fibronectin and increases *L. monocytogenes* adhesion to eukaryotic cells in the presence of exogenous fibronectin. FbpA is secreted by the SecA2 pathway and exposed on the bacterial surface. In addition to its fibronectin-binding capacity, FbpA coprecipitates with the virulence factors listeriolysin O (LLO) and InlB. Expression of FbpA modulates the protein levels of LLO and InlB, suggesting that it could function as a chaperone to prevent the degradation of virulence factors (Dramsi *et al.*, 2004).

4.1.4. Flagella

L. monocytogenes produces up to six peritrichous flagella (Leifson and Palen, 1955). Flagella are composed of a basal body, hook/junction proteins, a flagellar motor/switch, a flagella export apparatus, and a flagellar filament containing mostly the flagellin protein FlaA. Flagellin is a potent proinflammatory protein that activates Toll-like receptor (TLR) 5 (Hayashi *et al.*, 2001). Moreover, flagellin has been reported to have peptidoglycan

hydrolyzing activity (Popowska and Markiewicz, 2004). While many flagella are produced at 20 °C, the expression of flagellar motility genes is repressed at 37 °C (Griffin and Robbins, 1944; Grundling *et al.*, 2004; Peel *et al.*, 1988; Way *et al.*, 2004). However, the temperature control of flagellar motility is less stringent in some *L. monocytogenes* strains (Grundling *et al.*, 2004; Way *et al.*, 2004). Flagellin expression at 37 °C is maintained in 20% of clinical isolates (Bigot *et al.*, 2005; Way *et al.*, 2004). Flagella contribute to *L. monocytogenes* adhesion and invasion of epithelial cells. Indeed, the nonmotile *flaA* mutant, *fliF* and *fliI* mutants, lacking the basal body and the ATPase of the flagellar export apparatus, and the *cheYA* chemotaxis mutant are strongly impaired in adhesion and invasion (Bigot *et al.*, 2005; Dons *et al.*, 2004). It has recently been demonstrated that flagella do not function as adhesins but that flagella-dependent motility promotes *L. monocytogenes* invasion of epithelial cells (O'Neil and Marquis, 2006). The specific role of flagellar motility and flagellin in the infectious process is not completely understood. Liver and spleen colonization of a *flaA* deletion mutant has been shown to be similar to that of a parental strain expressing flagellin constitutively, after intravenous infection of mice (Way *et al.*, 2004). However, survival of the parental strain producing flagellin seemed to be decreased compared with that of the *flaA* mutant, 7 days after intragastric inoculation of mice. The LD50 of *fliF* and *fliI* mutants was very modestly affected compared with that of the EGDe wild-type strain after intravenous infection of Swiss mice (Bigot *et al.*, 2005). Interestingly, the survival of the wild-type strain was lower than that of the *fliF* mutant in the spleen of BALB/c mice, 3 days after intragastric infection. A similar observation was reported with a *flaA* mutant that was recovered in higher numbers than the wild-type strain from the spleen of BALB/c mice, 3 days after intragastric inoculation (Dons *et al.*, 2004). However, the fact that this difference was not detected at 1 or 7 days postinfection is puzzling. Recently, flagellin was shown to be required for intestinal and liver colonization in the early phase of murine listeriosis, between 4 and 18 h after intragastric inoculation (O'Neil and Marquis, 2006). It could be hypothesized that *L. monocytogenes* regulates flagella synthesis in time and space, producing flagella to colonize the gastrointestinal tract after ingestion and repressing their synthesis as a means of innate immune evasion at later stages of the infectious process.

4.2. Internalization

4.2.1. Internalin

The internalin is a protein of 800 amino acids encoded by the *inlA* gene. It is composed of a typical N-terminal signal sequence followed by 15 leucine-rich repeats (LRRs) of 22 amino acids, a conserved interrepeat region and a second repeat region, the B repeat region. The C-terminus displays the

sequence LPTTG, which is recognized by the sortase A, a transamidase that covalently links LPXTG-containing proteins to the peptidoglycan. This surface protein is an invasin that mediates internalization of *L. monocytogenes* in epithelial cells. It was identified by screening a bank of transposon mutants for reduced entry in Caco-2 cells (Gaillard *et al.*, 1991). The intercellular adhesion glycoprotein E-cadherin was subsequently identified as the internalin ligand using affinity chromatography (Mengaud *et al.*, 1996). If E-cadherin ectodomain is sufficient for adherence of *L. monocytogenes* to cells, the intracytoplasmic β-catenin-binding domain is required for entry (Lecuit *et al.*, 2000). Bacterial interaction with E-cadherin triggers actin polymerization mediated by β-catenin and α-catenin interaction, leading to membrane extension and internalization. Recently, ARHGAP10, a Rho-GAP domain protein that interacts with the small GTP-binding protein Arf6 and is a new ligand of α-catenin identified by a two-hybrid screen, has been shown to be critical for recruitment of α-catenin and bacterial entry (Sousa *et al.*, 2005). The internalin-dependent entry pathway requires several other proteins including myosin VIIA, Src, cortactin, and Arp2/3. The myosin VIIA, a molecular motor recruited at adherens junctions by the transmembrane protein vezatin, could contribute to the contractile force necessary for internalization of *L. monocytogenes* (Sousa *et al.*, 2004). The tyrosine kinase Src and the small GTPase Rac1 promote the recruitment of cortactin leading to activation of the actin nucleator Arp2/3 necessary for E-cadherin-mediated bacterial entry (Sousa *et al.*, 2007).

Although internalin plays a major role in bacterial internalization into specific cell lines, the protein had a minor contribution to virulence in the murine models that were first used, irrespective of the route of infection, that is intravenous or intragastric inoculations (Gaillard *et al.*, 1996). It was later shown that the mouse E-cadherin does not interact efficiently with InlA (Lecuit *et al.*, 1999). Indeed, the interaction requires recognition of the proline 16 of the first extracellular domain of E-cadherin as found in human or guinea pig E-cadherins. However, the murine E-cadherin has a glutamic acid at position 16. A transgenic mouse expressing the human E-cadherin in the intestine was created and used to demonstrate the major role of internalin in the specific crossing of the intestinal barrier by *L. monocytogenes* (Lecuit *et al.*, 2001). Recently, a strain of *L. monocytogenes* expressing an internalin with two amino acid substitutions allowing efficient binding to murine E-cadherine was created (Wollert *et al.*, 2007). This new strain could be a powerful tool to study listeriosis in nontransgenic mice, circumventing limitations, and problems inherent to humanized mice.

In addition to its established role in crossing of the intestinal barrier, InlA is involved in the crossing of the maternofetal barrier (Lecuit *et al.*, 2004). Internalin is required for *L. monocytogenes* entry into E-cadherin-expressing syncytiotrophoblasts and crossing of the trophoblastic barrier in human placental explants (Lecuit *et al.*, 2004). Interestingly, the InlA protein is

truncated in some *L. monocytogenes* isolates. Truncation of InlA has been involved in defective invasion capacity of *L. monocytogenes* isolates from healthy carriers (Olier *et al.*, 2003). An epidemiological survey demonstrated that a full-length InlA was produced by 96% of *L. monocytogenes* clinical isolates and only 65% of the strains isolated from food products (Jacquet *et al.*, 2004). Another study confirmed that *inlA* mutations leading to premature stop codons were common in food isolates but rare in clinical isolates (Nightingale *et al.*, 2005). These results strongly suggest that a functional internalin is a key determinant in the pathogenesis of human listeriosis.

4.2.2. InlB

InlB is a 630-amino acid protein encoded by the gene *inlB*, which is located directly downstream of *inlA* in a two-gene operon (Gaillard *et al.*, 1991). The operon is regulated by PrfA and absent from *L. innocua* (Dramsi *et al.*, 1993; Glaser *et al.*, 2001; Lingnau *et al.*, 1995; Milohanic *et al.*, 2003). In contrast to internalin, InlB is required for *L. monocytogenes* internalization into a wide range of cells including epithelial cells, endothelial cells, hepatocytes, and fibroblasts (Braun *et al.*, 1998; Dramsi *et al.*, 1995; Greiffenberg *et al.*, 1998; Parida *et al.*, 1998). The InlB protein displays a signal sequence followed by seven LRRs, a B repeat, and three C-terminal GW modules. The GW modules interact noncovalently with lipoteichoic acids mediating loose attachment of InlB to the bacterial cell wall (Jonquieres *et al.*, 1999). The LRR region of the protein is sufficient to allow entry of noninvasive *L. innocua* or latex beads into cells (Braun *et al.*, 1999). However, the GW modules enhance internalization triggered by the LRR region. Binding of InlB to cellular glycosaminoglycans by its GW modules is required for efficient invasion (Banerjee *et al.*, 2004; Jonquieres *et al.*, 2001; Marino *et al.*, 2000, 2002, 2004). The GW modules of InlB also interact with the receptor for the globular head domain of the complement component C1q, gC1qR (Braun *et al.*, 2000). This interaction is not sufficient to allow entry but cooperates with the hepatocyte growth factor, also known as the tyrosine kinase receptor Met, for invasion (Khelef *et al.*, 2006). Met has been identified as the main receptor of InlB (Shen *et al.*, 2000). Interaction of InlB and Met results in transient phosphorylation of Met (Shen *et al.*, 2000), and recruitment and phosphorylation of the adaptor proteins Cbl, Gab1, and Shc leading to activation of the PI3-kinase (Ireton *et al.*, 1996, 1999). The PI3-kinase converts PI(4,5)P2 into PI(3,4,5)P3, which results in successive activation of Rac and LIM kinase. The LIM kinase regulates the actin depolymerizing factor cofilin and thus internalization of *L. monocytogenes* (Bierne *et al.*, 2001). The WAVE complex, N-WASP, Ena/VASP, and the Arp2/3 complex are other key effectors of the Met signaling pathway that are important for cytoskeletal rearrangements necessary for InlB-mediated entry (Bierne *et al.*, 2005). It has been demonstrated that

InlB induces monoubiquitination of Met by the ubiquitin ligase Cbl resulting in endocytosis of Met (Veiga and Cossart, 2005). *L. monocytogenes* exploits the endocytic machinery to invade the cell (Bonazzi and Cossart, 2006; Veiga and Cossart, 2006). Indeed, bacterial internalization was shown to be dependent on major components of the endocytic machinery such as clathrin, dynamin, eps15, Grb2, CIN85, cortactin, and Hrs (Veiga and Cossart, 2005, 2006; Veiga *et al.*, 2007).

Activation of Met by InlB is species-specific (Khelef *et al.*, 2006). InlB activates human and murine Met but not guinea pig and rabbit Met. In mice, InlB contributes slightly to colonization of the liver and spleen. In contrast, a role for InlB in *L. monocytogenes* virulence could not be detected in guinea pigs and rabbits (Khelef *et al.*, 2006).

4.2.3. SrtA and SrtB

Surface proteins displaying a C-terminal LPXTG motif are covalently linked to the bacterial cell wall peptidoglycan by sortases. Analysis of *L. monocytogenes* genome sequence revealed the presence of two genes encoding sortases, *srtA* and *srtB* (Bierne *et al.*, 2002). SrtA anchors InlA and several other LPXTG proteins to the peptidoglycane (Bierne *et al.*, 2002; Garandeau *et al.*, 2002; Pucciarelli *et al.*, 2005). Consequently, the sortase A is necessary for efficient entry into epithelial cells (Bierne *et al.*, 2002; Garandeau *et al.*, 2002). Interestingly, it has been shown that in contrast to deletion of *inlA*, inactivation of *srtA* leads to impaired colonization of the liver and spleen of mice after intragastric inoculation (Bierne *et al.*, 2002). Thus, the sortase A could be required for the anchoring of additional LPXTG proteins involved in virulence.

In *L. monocytogenes*, SrtB anchors a small group of proteins and may recognize two different sorting motifs, NXZTN and NPKXZ (Pucciarelli *et al.*, 2005). Inactivation of *L. monocytogenes* SrtB does not affect virulence in mice after intravenous inoculation (Bierne *et al.*, 2004). One of SrtB substrate is SvpA (Bierne *et al.*, 2004), a surface protein first reported to be involved in bacterial escape from the phagosome of macrophages and in virulence (Borezee *et al.*, 2001). It was later shown that the *svpA-srtB* locus does not contribute to virulence in mice after intravenous inoculation, but is required for efficient colonization of the liver, spleen, and intestine of mice infected by the oral route (Newton *et al.*, 2005).

4.2.4. Auto

The gene *aut* was identified by a comparative genomic approach (Cabanes *et al.*, 2002, 2004; Glaser *et al.*, 2001). It is absent from the genome of the nonpathogenic species *L. innocua*. It encodes Auto, a surface protein of 572 amino acids. The N-terminus of the protein contains a signal sequence and

an autolysin domain. The C-terminus displays a cell wall attachment domain composed of four GW modules. Inactivation of Auto decreases bacterial entry into cells. However, expression of the autolysin in *L. innocua* does not confer invasivity. Thus, Auto is necessary but not sufficient for entry. The decreased invasive potential of the *aut* deletion mutant correlates with its attenuation *in vivo*. Indeed, Auto is required for *L. monocytogenes* virulence in mice infected intravenously and in guinea pigs after intragastric inoculation (Cabanes *et al.*, 2004). The precise function of Auto remains to be determined. The autolytic activity of the protein could possibly play a role in pathogenicity, for example, by controlling the composition and structure of the bacterial surface during the infectious process.

4.2.5. Vip

The gene encoding the surface protein Vip was also identified by comparative genomics of *Listeria* species (Cabanes *et al.*, 2002, 2005; Glaser *et al.*, 2001). PrfA regulates the expression of the gene *vip*, which is absent from the genome of *L. innocua* (Cabanes *et al.*, 2005). The Vip protein contains a C-terminal LPXTG motif and is anchored to the peptidoglycane by the sortase A (Cabanes *et al.*, 2005). Vip is required for invasion of several cell lines and contributes to virulence in mice infected intravenously. In contrast to InlA, it is required for virulence in mice after intragastric inoculation independently of the expression of human E-cadherin at the intestinal level. It is also an important determinant of virulence in the guinea pig. The endoplasmic reticulum resident chaperone Gp96 has been identified as a ligand of Vip (Cabanes *et al.*, 2005). Recently, the creation of a macrophage-specific gp96-deficient mouse allowed to establish that Gp96 is an important chaperone for all TLRs that have been tested (Yang *et al.*, 2007). Interestingly, these gp96-deficient mice were highly susceptible to listeriosis. In wild-type mice, interaction of Vip with Gp96 could possibly interfere with TLRs trafficking resulting in the control of the innate immune response by *L. monocytogenes*.

4.2.6. LpeA

The *lpeA* gene encoding a 35-kDa lipoprotein was identified by analysis of *L. monocytogenes* genome sequence (Glaser *et al.*, 2001; Reglier-Poupet *et al.*, 2003b). The LpeA (for lipoprotein promoting entry) protein is homologous to PsaA, a lipoprotein involved in *Streptococcus pneumoniae* adherence to cells. LpeA is not involved in adherence but is required for entry of *L. monocytogenes* into nonprofessional phagocytic cells. However, the impaired invasion of an *lpeA* mutant is not correlated with a decrease in virulence in mice (Reglier-Poupet *et al.*, 2003b).

4.3. Vacuolar escape, intracellular survival and multiplication

4.3.1. Listeriolysin O

Listeriolysin O (LLO) is one of the major virulence determinants of *L. monocytogenes* (Kayal and Charbit, 2006; Schnupf and Portnoy, 2007; Vazquez-Boland *et al.*, 2001b). The *hly* gene encoding LLO was the first virulence gene identified in *Listeria*. Identification was based on transposon mutagenesis. Characterization of the *hly* genomic locus led to identification of the *L. monocytogenes* main virulence gene cluster composed of *prfA*, *plcA*, *hly*, *mpl*, *actA*, *plcB*, and *orfX*. LLO is a secreted protein that belongs to the cholesterol-dependent cytolysin (CDC) toxin family. It is responsible for bacterial escape from primary and secondary vacuoles (Gedde *et al.*, 2000; Portnoy *et al.*, 1988). *L. monocytogenes* mutants lacking LLO fail to reach the cytoplasm and are nonvirulent (Cossart *et al.*, 1989; Gaillard *et al.*, 1986, 1987; Kathariou *et al.*, 1987; Portnoy *et al.*, 1988). The activity of LLO is optimal at the acidic pH of the phagosome. It is less active at the neutral pH of the cytoplasm, preventing excessive cell damage. LLO binds to the cell plasma membrane as monomers that oligomerize into large complexes that penetrate the membrane and contribute to pore formation. As other CDCs, LLO is a potent signaling protein that can activate important signaling pathways such as NF-κB (Kayal *et al.*, 1999), MAP kinase (Tang *et al.*, 1996), and protein kinase C (Wadsworth and Goldfine, 2002) and induce proinflammatory cytokine secretion (Kayal *et al.*, 1999). Interestingly, LLO is also required for *L. monocytogenes* entry into cells (Dramsi and Cossart, 2003). The specific functions of LLO in the signaling and entry processes remain to be elucidated.

4.3.2. Phospholipases

L. monocytogenes secretes two phospholipases C (PLC), PlcA and PlcB, involved in the bacterial escape from the vacuoles (Goldfine *et al.*, 1998). PlcA is a secreted phosphatidylinositol-specific PLC (PI-PLC) encoded by the *plcA* gene (Leimeister-Wachter *et al.*, 1991; Mengaud *et al.*, 1991). PlcB is a secreted phosphatidylcholine PLC (PC-PLC) of broad substrate range encoded by the *plcB* gene (Geoffroy *et al.*, 1991; Vazquez-Boland *et al.*, 1992). PlcB is expressed as a proenzyme. The zinc metalloprotease encoded by the gene *mpl* is required for maturation of PlcB (Domann *et al.*, 1991; Raveneau *et al.*, 1992). The two phospholipases act in synergy with LLO to lyze primary and secondary vacuoles allowing *L. monocytogenes* to escape into the cytoplasm (Camilli *et al.*, 1993; Grundling *et al.*, 2003; Smith *et al.*, 1995). PlcB can also promote lysis of the primary vacuole in the absence of LLO (Grundling *et al.*, 2003; Marquis *et al.*, 1995). Both phospholipases are required for virulence in mice (Camilli *et al.*, 1991, 1993; Raveneau *et al.*, 1992; Schluter *et al.*, 1998; Smith *et al.*, 1995).

Recently, it has been demonstrated that *L. monocytogenes* phospholipases are necessary for evasion of autophagy (Birmingham *et al.*, 2007; Py *et al.*, 2007). Cellular invasion by *L. monocytogenes* first induces autophagy, a host degradative pathway important for both cell physiology and innate immunity. Expression of LLO is necessary for the induction of the autophagic response at the early time points after infection, suggesting a role for permeabilization of the vacuole in the induction of the degradative pathway. The expression PlcA and PlcB is then required for *L. monocytogenes* escape from autophagic degradation in nonprofessional phagocytic cells and macrophages (Birmingham *et al.*, 2007; Py *et al.*, 2007). The phospholipases may prevent autophagic killing by mediating escape from the double-membrane autophagosome or by inhibiting recognition of the target of the degradative pathway.

4.3.3. Lsp

The signal peptidase II Lsp is responsible for the maturation of lipoproteins in *L. monocytogenes* (Desvaux and Hebraud, 2006; Reglier-Poupet *et al.*, 2003a). A deletion mutant of the *lsp* gene fails to process lipoproteins and has a reduced virulence. Interestingly, the expression of *lsp* is strongly induced in the phagosome of infected macrophages. This induction correlates with the important role of Lsp, and thus lipoprotein maturation, in *L. monocytogenes* escape from the phagosome (Reglier-Poupet *et al.*, 2003a).

4.3.4. SipX and SipZ

L. monocytogenes genome contains three contiguous type I signal peptidase genes, *sipX*, *sipY*, and *sipZ*, for cleavage of signal peptides proteins exported and secreted by the general secretory pathway (Bonnemain *et al.*, 2004; Desvaux and Hebraud, 2006). The expression of the three genes is induced in the phagosome of infected cells (Raynaud and Charbit, 2005). The signal peptidases SipX and SipZ are required for full virulence (Bonnemain *et al.*, 2004). In contrast, inactivation of SipY did not impaired *L. monocytogenes* virulence. In addition, SipZ is required for efficient secretion of LLO and PC-PLC. Consequently, inactivation of SipZ restricts bacterial intracellular multiplication (Bonnemain *et al.*, 2004).

4.3.5. Hpt

Once free in the cytoplasm, *L. monocytogenes* expresses specific determinants to acquire nutrients necessary for intracellular multiplication. Uptake of glucose-1-phosphate, a source of carbon and energy available in the cytosol, depends on the PrfA-regulated hexose phosphate transporter Hpt (Chico-Calero *et al.*, 2002). Interestingly, Hpt is a structural and functional homologue of the eukaryotic glucose-6-phosphate translocase required for transport of glucose-6-phosphate from the cytosol into the endoplasmic

reticulum. Hpt has been shown to be required for intracellular replication of *L. monocytogenes* and for virulence in mice (Chico-Calero *et al.*, 2002).

4.3.6. LplA1

L. monocytogenes is a lipoate auxotroph. In order to scavenge this important cofactor, bacteria produce lipoate ligases to lipoylate specific metabolic enzymes. Analysis of *L. monocytogenes* genome sequence reveals two genes encoding putative lipoate ligases, *lplA1* and *lplA2* (Keeney *et al.*, 2007). However, only *lplA1* is required for intracellular replication and virulence (Keeney *et al.*, 2007; O'Riordan *et al.*, 2003). LplA1 is critical for utilization of host lipoyl peptides as a source of lipoate by *L. monocytogenes*.

4.3.7. Fri

L. monocytogenes genome encodes a single ferritin, Fri, which is involved in iron storage. Expression of the *fri* gene is controlled by the hydrogen peroxide regulator PerR and sigma B (Olsen *et al.*, 2005). The ferritin is required for protection against reactive oxygen species and contributes to *L. monocytogenes* survival and replication in macrophages and nonprofessional phagocytic cells (Dussurget *et al.*, 2005; Mohamed *et al.*, 2006; Olsen *et al.*, 2005). The impaired survival of a *fri* deletion mutant in macrophages correlates with decreased virulence of the mutant in mice (Dussurget *et al.*, 2005; Mohamed *et al.*, 2006; Olsen *et al.*, 2005). The capacity to prevent excessive production of reactive oxygen species and control the level of iron is an important component of *L. monocytogenes* intracellular survival strategy.

4.3.8. HupC

L. monocytogenes does not secrete siderophores but can use siderophores from other microorganisms or transferrin, hemin, and hemoglobin to obtain iron (Jin *et al.*, 2006; Newton *et al.*, 2005; Simon *et al.*, 1995). The permease HupC is an ABC transporter required for hemin and hemoglobin uptake (Jin *et al.*, 2006; Newton *et al.*, 2005). The LD50 of a mutant *L. monocytogenes* lacking *hupC* was strongly increased in Swiss mice infected intravenously, suggesting that acquisition of iron from blood or other infected sites facilitates *L. monocytogenes* host colonization.

4.3.9. MnSOD

SOD plays an important role in protection against oxidative stress and has been shown to contribute to the pathogenic potential of many bacterial species. *L. monocytogenes* produces a single MnSOD encoded by the gene *sod* (Archambaud *et al.*, 2006; Brehm *et al.*, 1992; Glaser *et al.*, 2001). A *sod* deletion mutant is impaired in survival within macrophages and in virulence in mice (Archambaud *et al.*, 2006). Cytoplasmic MnSOD is phosphorylated on serine and threonine residues and can be dephosphorylated by the serine/threonine phosphatase Stp resulting in an increased SOD activity

(Archambaud *et al.*, 2006). *L. monocytogenes* MnSOD is the first bacterial SOD shown to be regulated by phosphorylation. The most active nonphosphorylated form of MnSOD is secreted via the SecA2 pathway in infected cells where it can protect *L. monocytogenes* from reactive oxygen species. Interestingly, the MnSOD becomes phosphorylated in the host cell by a putative host kinase that could control the enzyme activity (Archambaud *et al.*, 2006), suggesting a new innate mechanism of the cell to counteract an important bacterial determinant of the infectious process.

4.3.10. RelA

The *relA* gene encodes a (p)ppGpp synthetase. An *L. monocytogenes relA* transposon insertion mutant was unable to accumulate (p)ppGpp in response to amino acid starvation (Taylor *et al.*, 2002). The virulence of the mutant was strongly attenuated in mice, indicating an essential role of the stringent response in the survival and multiplication of *L. monocytogenes* in the host. Recently, RelA has been shown to be important for bacterial growth in macrophages and nonprofessional phagocytic cells, suggesting that the ability of *L. monocytogenes* to mount a stringent response is required for efficient intracellular multiplication (Bennett *et al.*, 2007).

4.3.11. Lgt

The lipoprotein diacylglyceryl transferase Lgt catalyzes transfer of an *N*-acyl diglyceride group from a glycerophospholipid to the sulfhydryl moiety of a cysteine residue conserved in the signal peptides of lipoprotein precursors. The product of the reaction is then cleaved by the signal peptidase Lsp. Deletion of *lgt* impairs intracellular growth of *L. monocytogenes* (Baumgartner *et al.*, 2007), confirming the importance of lipoprotein processing for pathogenicity (Reglier-Poupet *et al.*, 2003a).

4.4. Cell–cell spread

4.4.1. ActA

After synthesis of the determinants responsible for entry, intracellular survival, lysis of the vacuole, and cytosolic replication, *L. monocytogenes* induces polymerization of actin filaments to move in the cytoplasm and to spread from cell to cell (Mounier *et al.*, 1990; Theriot *et al.*, 1992; Tilney and Portnoy, 1989; Tilney *et al.*, 1990). The surface protein ActA is the only bacterial determinant necessary for actin-based motility of *L. monocytogenes* (Fig. 1.2) (Domann *et al.*, 1992; Kocks *et al.*, 1992). Indeed, *L. innocua* expressing ActA and latex beads coated with ActA acquire the capacity to polymerize actin and move (Cameron *et al.*, 1999; Kocks *et al.*, 1995). ActA is one of the major virulence determinants of *L. monocytogenes* (Domann *et al.*, 1992). ActA is a protein of 639 amino acids containing an N-terminal signal sequence and a C-terminal transmembrane domain (Domann *et al.*,

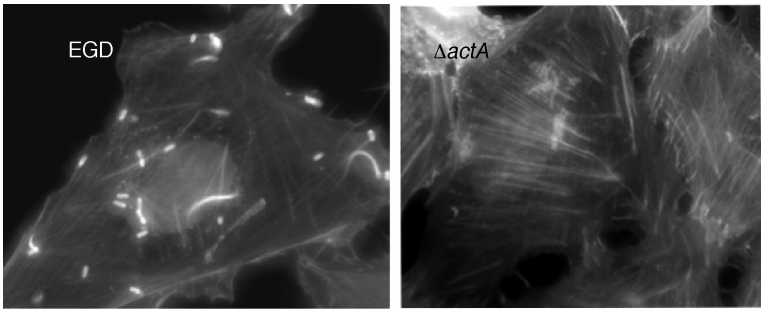

Figure 1.2 Vero cells infected with *L. monocytogenes* EGD (left panel) or its isogenic *actA* mutant (right panel). Cells were processed for triple fluorescence microscopy 5 h after infection. Bacteria were labeled with a polyclonal anti-*Listeria* antibody (black), actin with FITC-phalloidin (dark gray), and nuclei with DAPI (light gray). Actin accumulates around the parental strain EGD, leading to the formation of typical comet tails. In cells infected with the *actA* mutant, bacteria are unable to induce actin polymerization and multiply in the cytoplasm forming perinuclear microcolonies. (See Color Insert.)

1992; Kocks *et al.*, 1992). The central part of the protein presents a domain composed of four proline-rich repeats that binds proteins of the Ena/VASP family, which modulate speed and directionality of bacterial movement (Auerbuch *et al.*, 2003; Chakraborty *et al.*, 1995; Geese *et al.*, 2002; Lasa *et al.*, 1995; Laurent *et al.*, 1999; Niebuhr *et al.*, 1997). The N-terminal region of ActA is sufficient to induce motility (Lasa *et al.*, 1997). It binds and activates the Arp2/3 complex inducing actin polymerization, mimicking proteins of the WASP family (Boujemaa-Paterski *et al.*, 2001; Skoble *et al.*, 2000, 2001). Actin tails induced by *L. monocytogenes* are composed of branched filaments similar to those of *Shigella flexneri*, in contrast to *Rickettsia conorii* actin tails which contain long and unbranched filaments (Gouin *et al.*, 1999, 2004, 2005).

ActA is also involved in cell attachment and entry by recognition of heparan sulfate (Alvarez-Dominguez *et al.*, 1997). Inactivation of ActA impairs *L. monocytogenes* invasion in macrophages and epithelial cells (Alvarez-Dominguez *et al.*, 1997; Suarez *et al.*, 2001). In addition, expression of ActA in *L. innocua* is sufficient to confer the capacity to enter epithelial cells (Suarez *et al.*, 2001).

A third role has been assigned to ActA in preventing bacterial autophagy in the cytosol of macrophages (Birmingham *et al.*, 2007; Rich *et al.*, 2003). Some *L. monocytogenes* are targeted by autophagy during early stages of infection by an LLO-dependent process. ActA expression is sufficient to promote autophagy evasion in the cytosol at later stages of infection (Birmingham *et al.*, 2007). ActA could possibly lead to escape from autophagy by actin-based movement or by actin masking of the bacteria, inhibiting recognition of autophagy targets.

4.4.2. SecA2

The auxiliary SecA paralogue protein SecA2 was identified by analysis of spontaneous rough variants of *L. monocytogenes*, which grew in chains (Lenz and Portnoy, 2002). In contrast to SecA, SecA2 is not essential for cell viability. SecA2 is required for virulence in mice and cell–cell spread in cultured cells (Lenz and Portnoy, 2002; Lenz *et al.*, 2003). Using a proteomic approach, 17 SecA2-dependent secreted and surface proteins were identified including the autolysin p60 and the *N*-acetylmuramidase NamA (Lenz *et al.*, 2003). These two peptidoglycane hydrolases and other SecA2 targets, such FbpA (Dramsi *et al.*, 2004) and MnSOD (Archambaud *et al.*, 2006), are important determinants of the infectious process. Thus, SecA2 could have evolved in part to mediate secretion of a subset of proteins contributing to virulence.

5. Immunomodulation and Persistence

5.1. Evasion and manipulation of host immune response

5.1.1. PgdA

Bacterial cell wall peptidoglycan is the pathogen-associated molecular pattern detected by the nucleotide-binding oligomerization domain (NOD) protein family of pattern-recognition receptors, resulting in activation of the NF-κB pathway (Chamaillard *et al.*, 2003; Girardin *et al.*, 2003a,b, Inohara *et al.*, 2003). Analysis of *L. monocytogenes* peptidoglycan revealed deacetylation of *N*-acetylglucosamine residues (Boneca *et al.*, 2007; Kamisango *et al.*, 1982). *L. monocytogenes* genome contains a single peptidoglycane *N*-deacetylase gene, *pgdA* (Boneca *et al.*, 2007; Glaser *et al.*, 2001). Inactivation of *pgdA* dramatically increases *L. monocytogenes* sensitivity to lyzozyme *in vitro* and strongly attenuates virulence in mice infected intravenously and in transgenic mice expressing human E-cadherin after intragastric inoculation (Boneca *et al.*, 2007). PgdA is required for survival within macrophage vacuoles (Fig. 1.3) and prevents proinflammatory cytokine and interferon-β secretion (Boneca *et al.*, 2007). Thus, peptidoglycan *N*-deacetylation is critical for evasion of host innate defenses.

5.1.2. p60

The autolysin p60, also known as the invasion-associated protein Iap or the cell wall hydrolase A CwhA, is a 60-kDa protein secreted by the SecA2 pathway. This peptidoglycan hydrolase promotes *L. monocytogenes* infection *in vivo* (Faith *et al.*, 2007; Lenz *et al.*, 2003). The mechanism of virulence attenuation of p60-deficient mutants is not completely understood. Recently, the reduced capacity of a p60 mutant to cause systemic infection

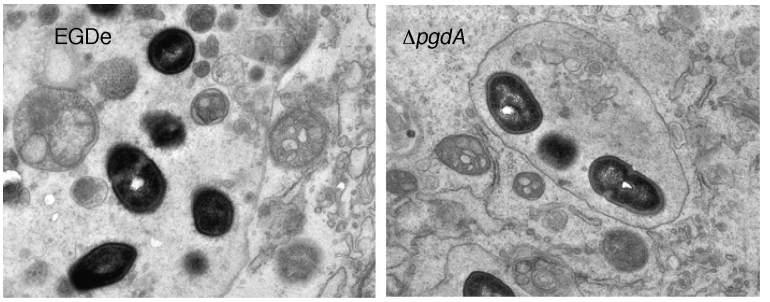

Figure 1.3 RAW264.7 macrophages infected with *L. monocytogenes* EGDe (left panel) or the *pgdA* deletion mutant (right panel). Cells were processed for electron microscopy 4 h after infection. Impaired survival of the *pgdA* mutant was correlated with delay in escape from the phagosome compared with the parental strain that was free in the cytoplasm.

of mice after intragastric inoculation was correlated to a diminished ability to enter and multiply within epithelial cells (Faith *et al.*, 2007). Interestingly, p60 has also been shown to indirectly increase NK cell activation and interferon-γ production (Humann *et al.*, 2007). It was suggested that p60 could promote early bacterial multiplication by subversion of interferon-γ-mediated immune responses and manipulation of deleterious and protective effects of interferon-γ production. The bacterial components that are released by the catalytic activity of p60 and directly modulate host innate response remain to be identified.

5.1.3. LLO

L. monocytogenes infection leads to modulation of expression of host genes. Posttranslational modifications of histones play an essential role in chromatin remodeling and gene expression regulation. It has been shown that infection of human endothelial cells by *L. monocytogenes* induces a p38 MAPK and MEK1-dependent acetylation of histone H4 and phosphorylation and acetylation of histone H3 globally as well as specifically at the promoter of IL8 (Schmeck *et al.*, 2005). LLO is required for upregulation of adhesion molecules and chemokines in endothelial cells infected by *L. monocytogenes* (Kayal *et al.*, 1999). Recently, LLO was shown to be critical for dephosphorylation of histone H3 and deacetylation of histone H4 during early phase of infection (Hamon *et al.*, 2007). Indeed, decreased LLO-mediated histone modifications were associated to modulation of host cell gene expression (Hamon *et al.*, 2007). Interestingly, transcription of the chemokine gene *cxcl2* and of other specific immunity genes was decreased, suggesting that LLO genetic reprogramming of the host cell could be an additional mechanism by which *L. monocytogenes* manipulate the host immune response.

5.1.4. MprF

L. monocytogenes multiple peptide resistance factor MprF is a membrane protein of 98 kDa regulated by the response regulator VirR (Mandin *et al.*, 2005; Thedieck *et al.*, 2006). MprF is required for synthesis of lysylphosphatidylglycerol and for lysinylation of diphosphatidylglycerol, two-membrane phospholipids (Thedieck *et al.*, 2006). Inactivation of MprF results in a decreased invasivity in both epithelial cells and macrophages and in attenuation of the virulence in mice. MprF is critical for resistance to cationic antimicrobial peptides and could be another mechanism of *L. monocytogenes* to escape host innate immune response.

5.2. Persistence

L. monocytogenes is a common transient colonizer of the human gastrointestinal tract that does not cause invasive disease unless a combination of host susceptibility factors, bacterial virulence determinants, and a high infective dose is met. Asymptomatic fecal carriage in healthy individuals has a prevalence of 2–10% (Schlech, 2000). The mechanisms used by *L. monocytogenes* to persist in the host are not fully understood.

L. monocytogenes infection of the gallbladder has been documented in humans (Allerberger *et al.*, 1989; Gluck *et al.*, 2002; Gordon and Singer, 1986; Loupa *et al.*, 2007). In addition, *L. monocytogenes* was isolated from liver, bile, and feces of mice inoculated subcutaneously, suggesting that bacteria reached the intestine by biliary excretion (Briones *et al.*, 1992). *L. monocytogenes* can replicate extracellularly in the gallbladder of mice after oral or intravenous inoculation (Hardy *et al.*, 2004). Bacteria growing in the lumen of the gallbladder can transit through the bile duct into the intestine as soon as 5 min after induction of gallbladder contraction by food or cholecystokinin (Hardy *et al.*, 2006). Bacteria then move through the intestinal lumen, are excreted in the environment, and possibly reinfect mice. *L. monocytogenes* strains causing human disease express a BSH conferring resistance to bile antimicrobial activity and the capacity to colonize the gastrointestinal tract (Dussurget *et al.*, 2002). *L. monocytogenes* is particularly well equipped to survive in presence of bile as several other important genetic loci involved in bile resistance have been identified (Begley *et al.*, 2002, 2003, 2005; Sleator *et al.*, 2005). Thus, gallbladder could represent a niche where *L. monocytogenes* grows in the absence of commensal competitors and specific immune response. Dissemination of *L. monocytogenes* from the gallbladder to the intestine and the environment could play an important role in transient or chronic shedding and in transmission.

6. Virulence Determinants of Unknown Function

6.1. InlC

InlC (also designated internalin-related protein A, IrpA) is a secreted protein of 297 amino acids containing a central region composed of 6 LRRs followed by a C-terminal Ig-like domain (Domann *et al.*, 1997; Engelbrecht *et al.*, 1996; Ooi *et al.*, 2006). The *inlC* gene, which is absent from the genome of *L. innocua*, is transcribed by PrfA-dependent and -independent mechanisms (Domann *et al.*, 1997; Luo *et al.*, 2004). InlC contributes to *L. monocytogenes* virulence in mice (Domann *et al.*, 1997; Engelbrecht *et al.*, 1996). The expression of *inlC* is strongly induced in the cytoplasm of infected macrophages (Engelbrecht *et al.*, 1996). However, deletion of *inlC* does not affect invasion, intracellular survival, or cell spread (Domann *et al.*, 1997; Engelbrecht *et al.*, 1996; Greiffenberg *et al.*, 1998). The function and binding partners of InlC have yet to be discovered.

6.2. InlGHE

A gene cluster encoding the three internalins InlG, InlH, and InlE has been identified in some *L. monocytogenes* strains (Raffelsbauer *et al.*, 1998). An in-frame deletion of the *inlGHE* operon had no effect on cellular invasion and its function remains unknown. However, the mutant showed reduced colonization of the spleen and liver after infection of mice by the oral route (Raffelsbauer *et al.*, 1998). A specific role for InlH in virulence was later demonstrated in mice infected intravenously (Schubert *et al.*, 2001).

6.3. InlJ

Another internalin encoding gene, *inlJ*, was identified by analyzing *L. monocytogenes* genome sequence (Cabanes *et al.*, 2002; Glaser *et al.*, 2001; Sabet *et al.*, 2005). InlJ is required for full virulence of *L. monocytogenes* in mice infected intravenously and after intragastric inoculation in transgenic mice expressing the human E-cadherin at the level of the intestine (Sabet *et al.*, 2005). However, inactivation of *inlJ* does not affect *L. monocytogenes* capacity to infect cells. The function of this internalin remains to be determined.

7. Conclusion

The advent of comparative genomics and transcriptomic technologies allowing analysis of host cell and bacterial gene expression during the infectious cycle coupled to the development of new animal models of

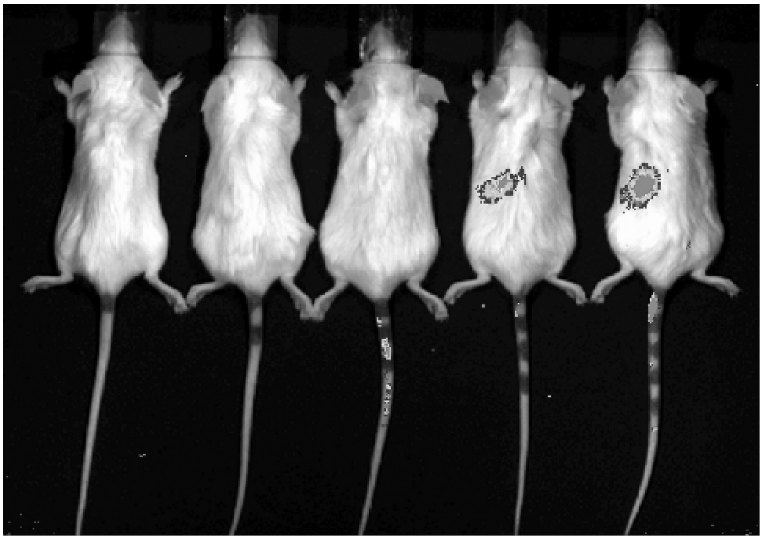

Figure 1.4 **Noninvasive bioluminescence imaging of listeriosis in BALB/c mice. Bioluminescent splenic signals corresponding to bacterial replication foci were detected 48 h after intravenous inoculation of 8×10^3, 4×10^4, 2×10^5, 10^6, and 5×10^6 *L. monocytogenes* from left to right. (See Color Insert.)**

infection have greatly improved our knowledge of *L. monocytogenes* pathogenesis. Here, we have highlighted some of the important bacterial determinants that have been involved in the infectious process. However, our understanding of listeriosis is still far from complete. As more virulence determinants are identified, determination of their specific function, their host partners, and where and when they are expressed during the infectious process will become the next challenge. Identification of the key components of host immune response involved in listeriosis and how they can be manipulated by *L. monocytogenes* should benefit from the recent advances in the field of innate immunity. Dynamic gene profiling *in vivo*, noninvasive imaging in relevant animal models (Fig. 1.4), and real-time imaging in living cells will surely help to address the complexity of *L. monocytogenes* interactions with the host and bring us a step closer to a comprehensive understanding of the disease.

ACKNOWLEDGMENTS

I apologize to authors whose relevant work could not be cited owing to space limitations. Past and present colleagues of the Cossart laboratory and the *Listeria* genome consortium are gratefully acknowledged for contributing to the work described in this chapter and for many valuable discussions over the last years. Research in the laboratory is supported by the Pasteur Institute, INRA, Inserm, ANR, EU FP6, and HHMI.

REFERENCES

Abachin, E., Poyart, C., Pellegrini, E., Milohanic, E., Fiedler, F., Berche, P., and Trieu-Cuot, P. (2002). Formation of D-alanyl-lipoteichoic acid is required for adhesion and virulence of *Listeria monocytogenes*. *Mol. Microbiol.* **43,** 1–14.

Allerberger, F., Langer, B., Hirsch, O., Dierich, M. P., and Seeliger, H. P. (1989). *Listeria monocytogenes* cholecystitis. *Z. Gastroenterol.* **27,** 145–147.

Alvarez-Dominguez, C., Vazquez-Boland, J. A., Carrasco-Marin, E., Lopez-Mato, P., and Leyva-Cobian, F. (1997). Host cell heparan sulfate proteoglycans mediate attachment and entry of *Listeria monocytogenes*, and the listerial surface protein ActA is involved in heparan sulfate receptor recognition. *Infect. Immun.* **65,** 78–88.

Archambaud, C., Gouin, E., Pizarro-Cerda, J., Cossart, P., and Dussurget, O. (2005). Translation elongation factor EF-Tu is a target for Stp, a serine-threonine phosphatase involved in virulence of *Listeria monocytogenes*. *Mol. Microbiol.* **56,** 383–396.

Archambaud, C., Nahori, M. A., Pizarro-Cerda, J., Cossart, P., and Dussurget, O. (2006). Control of *Listeria* superoxide dismutase by phosphorylation. *J. Biol. Chem.* **281,** 31812–31822.

Auerbuch, V., Loureiro, J. J., Gertler, F. B., Theriot, J. A., and Portnoy, D. A. (2003). Ena/VASP proteins contribute to *Listeria monocytogenes* pathogenesis by controlling temporal and spatial persistence of bacterial actin-based motility. *Mol. Microbiol.* **49,** 1361–1375.

Autret, N., Raynaud, C., Dubail, I., Berche, P., and Charbit, A. (2003). Identification of the agr locus of *Listeria monocytogenes*: Role in bacterial virulence. *Infect. Immun.* **71,** 4463–4471.

Banerjee, M., Copp, J., Vuga, D., Marino, M., Chapman, T., van der Geer, P., and Ghosh, P. (2004). GW domains of the *Listeria monocytogenes* invasion protein InlB are required for potentiation of Met activation. *Mol. Microbiol.* **52,** 257–271.

Baumgartner, M., Karst, U., Gerstel, B., Loessner, M., Wehland, J., and Jansch, L. (2007). Inactivation of Lgt allows systematic characterization of lipoproteins from *Listeria monocytogenes*. *J. Bacteriol.* **189,** 313–324.

Begley, M., Gahan, C. G., and Hill, C. (2002). Bile stress response in *Listeria monocytogenes* LO28: Adaptation, cross-protection, and identification of genetic loci involved in bile resistance. *Appl. Environ. Microbiol.* **68,** 6005–6012.

Begley, M., Hill, C., and Gahan, C. G. (2003). Identification and disruption of btlA, a locus involved in bile tolerance and general stress resistance in *Listeria monocytogenes*. *FEMS Microbiol. Lett.* **218,** 31–38.

Begley, M., Sleator, R. D., Gahan, C. G., and Hill, C. (2005). Contribution of three bile-associated loci, bsh, pva, and btlB, to gastrointestinal persistence and bile tolerance of *Listeria monocytogenes*. *Infect. Immun.* **73,** 894–904.

Bennett, H. J., Pearce, D. M., Glenn, S., Taylor, C. M., Kuhn, M., Sonenshein, A. L., Andrew, P. W., and Roberts, I. S. (2007). Characterization of relA and codY mutants of *Listeria monocytogenes*: Identification of the CodY regulon and its role in virulence. *Mol. Microbiol.* **63,** 1453–1467.

Bierne, H., and Cossart, P. (2007). *Listeria monocytogenes* surface proteins: From genome predictions to function. *Microbiol. Mol. Biol. Rev.* **71,** 377–397.

Bierne, H., Gouin, E., Roux, P., Caroni, P., Yin, H. L., and Cossart, P. (2001). A role for cofilin and LIM kinase in *Listeria*-induced phagocytosis. *J. Cell Biol.* **155,** 101–112.

Bierne, H., Mazmanian, S. K., Trost, M., Pucciarelli, M. G., Liu, G., Dehoux, P., Jansch, L., Garcia-del Portillo, F., Schneewind, O., and Cossart, P. (2002). Inactivation of the srtA gene in *Listeria monocytogenes* inhibits anchoring of surface proteins and affects virulence. *Mol. Microbiol.* **43,** 869–881.

Bierne, H., Garandeau, C., Pucciarelli, M. G., Sabet, C., Newton, S., Garcia-del Portillo, F., Cossart, P., and Charbit, A. (2004). Sortase B, a new class of sortase in *Listeria monocytogenes*. *J. Bacteriol.* **186,** 1972–1982.

Bierne, H., Miki, H., Innocenti, M., Scita, G., Gertler, F. B., Takenawa, T., and Cossart, P. (2005). WASP-related proteins, Abi1 and Ena/VASP are required for *Listeria* invasion induced by the Met receptor. *J. Cell Sci.* **118,** 1537–1547.

Bigot, A., Pagniez, H., Botton, E., Frehel, C., Dubail, I., Jacquet, C., Charbit, A., and Raynaud, C. (2005). Role of FliF and FliI of *Listeria monocytogenes* in flagellar assembly and pathogenicity. *Infect. Immun.* **73,** 5530–5539.

Birmingham, C. L., Canadien, V., Gouin, E., Troy, E. B., Yoshimori, T., Cossart, P., Higgins, D. E., and Brumell, J. H. (2007). *Listeria monocytogenes* evades killing by autophagy during colonization of host cells. *Autophagy* **3,** 442–451.

Bonazzi, M., and Cossart, P. (2006). Bacterial entry into cells: A role for the endocytic machinery. *FEBS Lett.* **580,** 2962–2967.

Boneca, I. G., Dussurget, O., Cabanes, D., Nahori, M. A., Sousa, S., Lecuit, M., Psylinakis, E., Bouriotis, V., Hugot, J. P., Giovannini, M., Coyle, A., Bertin, J., *et al.* (2007). A critical role for peptidoglycan *N*-deacetylation in *Listeria* evasion from the host innate immune system. *Proc. Natl. Acad. Sci. USA* **104,** 997–1002.

Bonnemain, C., Raynaud, C., Reglier-Poupet, H., Dubail, I., Frehel, C., Lety, M. A., Berche, P., and Charbit, A. (2004). Differential roles of multiple signal peptidases in the virulence of *Listeria monocytogenes*. *Mol. Microbiol.* **51,** 1251–1266.

Borezee, E., Pellegrini, E., and Berche, P. (2000). OppA of *Listeria monocytogenes*, an oligopeptide-binding protein required for bacterial growth at low temperature and involved in intracellular survival. *Infect. Immun.* **68,** 7069–7077.

Borezee, E., Pellegrini, E., Beretti, J. L., and Berche, P. (2001). SvpA, a novel surface virulence-associated protein required for intracellular survival of *Listeria monocytogenes*. *Microbiology* **147,** 2913–2923.

Boujemaa-Paterski, R., Gouin, E., Hansen, G., Samarin, S., Le Clainche, C., Didry, D., Dehoux, P., Cossart, P., Kocks, C., Carlier, M. F., and Pantaloni, D. (2001). *Listeria* protein ActA mimics WASp family proteins: It activates filament barbed end branching by Arp2/3 complex. *Biochemistry* **40,** 11390–11404.

Braun, L., Ohayon, H., and Cossart, P. (1998). The InIB protein of *Listeria monocytogenes* is sufficient to promote entry into mammalian cells. *Mol. Microbiol.* **27,** 1077–1087.

Braun, L., Nato, F., Payrastre, B., Mazie, J. C., and Cossart, P. (1999). The 213-amino-acid leucine-rich repeat region of the *Listeria monocytogenes* InlB protein is sufficient for entry into mammalian cells, stimulation of PI 3-kinase and membrane ruffling. *Mol. Microbiol.* **34,** 10–23.

Braun, L., Ghebrehiwet, B., and Cossart, P. (2000). gC1q-R/p32, a C1q-binding protein, is a receptor for the InlB invasion protein of *Listeria monocytogenes*. *EMBO J.* **19,** 1458–1466.

Brehm, K., Haas, A., Goebel, W., and Kreft, J. (1992). A gene encoding a superoxide dismutase of the facultative intracellular bacterium *Listeria monocytogenes*. *Gene* **118,** 121–125.

Briones, V., Blanco, M. M., Marco, A., Prats, N., Fernandez-Garayzabal, J. F., Suarez, G., Domingo, M., and Dominguez, L. (1992). Biliary excretion as possible origin of *Listeria monocytogenes* in fecal carriers. *Am. J. Vet. Res.* **53,** 191–193.

Buchrieser, C. (2007). Biodiversity of the species *Listeria monocytogenes* and the genus *Listeria*. *Microbes Infect.* **9,** 1147–1155.

Cabanes, D., Dehoux, P., Dussurget, O., Frangeul, L., and Cossart, P. (2002). Surface proteins and the pathogenic potential of *Listeria monocytogenes*. *Trends Microbiol* **10,** 238–245.

Cabanes, D., Dussurget, O., Dehoux, P., and Cossart, P. (2004). Auto, a surface associated autolysin of *Listeria monocytogenes* required for entry into eukaryotic cells and virulence. *Mol. Microbiol.* **51,** 1601–1614.

Cabanes, D., Sousa, S., Cebria, A., Lecuit, M., Garcia-del Portillo, F., and Cossart, P. (2005). Gp96 is a receptor for a novel *Listeria monocytogenes* virulence factor, Vip, a surface protein. *EMBO J.* **24,** 2827–2838.

Cameron, L. A., Footer, M. J., van Oudenaarden, A., and Theriot, J. A. (1999). Motility of ActA protein-coated microspheres driven by actin polymerization. *Proc. Natl. Acad. Sci. USA* **96,** 4908–4913.

Camilli, A., Goldfine, H., and Portnoy, D. A. (1991). *Listeria monocytogenes* mutants lacking phosphatidylinositol-specific phospholipase C are avirulent. *J. Exp. Med.* **173,** 751–754.

Camilli, A., Tilney, L. G., and Portnoy, D. A. (1993). Dual roles of plcA in *Listeria monocytogenes* pathogenesis. *Mol. Microbiol.* **8,** 143–157.

Chakraborty, T., Ebel, F., Domann, E., Niebuhr, K., Gerstel, B., Pistor, S., Temm-Grove, C. J., Jockusch, B. M., Reinhard, M., Walter, U., and Wehland, J. (1995). A focal adhesion factor directly linking intracellularly motile *Listeria monocytogenes* and *Listeria ivanovii* to the actin-based cytoskeleton of mammalian cells. *EMBO J.* **14,** 1314–1321.

Chamaillard, M., Hashimoto, M., Horie, Y., Masumoto, J., Qiu, S., Saab, L., Ogura, Y., Kawasaki, A., Fukase, K., Kusumoto, S., Valvano, M. A., Foster, S. J., *et al.* (2003). An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat. Immunol.* **4,** 702–707.

Chastanet, A., Derre, I., Nair, S., and Msadek, T. (2004). clpB, a novel member of the *Listeria monocytogenes* CtsR regulon, is involved in virulence but not in general stress tolerance. *J. Bacteriol.* **186,** 1165–1174.

Chatterjee, S. S., Hossain, H., Otten, S., Kuenne, C., Kuchmina, K., Machata, S., Domann, E., Chakraborty, T., and Hain, T. (2006). Intracellular gene expression profile of *Listeria monocytogenes*. *Infect. Immun.* **74,** 1323–1338.

Chico-Calero, I., Suarez, M., Gonzalez-Zorn, B., Scortti, M., Slaghuis, J., Goebel, W., and Vazquez-Boland, J. A. (2002). Hpt, a bacterial homolog of the microsomal glucose-6-phosphate translocase, mediates rapid intracellular proliferation in *Listeria*. *Proc. Natl. Acad. Sci. USA* **99,** 431–436.

Christiansen, J. K., Larsen, M. H., Ingmer, H., Sogaard-Andersen, L., and Kallipolitis, B. H. (2004). The RNA-binding protein Hfq of *Listeria monocytogenes*: Role in stress tolerance and virulence. *J. Bacteriol.* **186,** 3355–3362.

Cossart, P. (2007). Listeriology (1926–2007): The rise of a model pathogen. *Microbes Infect.* **9,** 1143–1146.

Cossart, P., Vicente, M. F., Mengaud, J., Baquero, F., Perez-Diaz, J. C., and Berche, P. (1989). Listeriolysin O is essential for virulence of *Listeria monocytogenes*: Direct evidence obtained by gene complementation. *Infect. Immun.* **57,** 3629–3636.

Cossart, P., Boquet, P., Normark, S., and Rappuoli, R. (1996). Cellular microbiology emerging. *Science* **271,** 315–316.

Cotter, P. D., Emerson, N., Gahan, C. G., and Hill, C. (1999). Identification and disruption of lisRK, a genetic locus encoding a two-component signal transduction system involved in stress tolerance and virulence in *Listeria monocytogenes*. *J. Bacteriol.* **181,** 6840–6843.

Cotter, P. D., Gahan, C. G., and Hill, C. (2001). A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. *Mol. Microbiol.* **40,** 465–475.

Cotter, P. D., Ryan, S., Gahan, C. G., and Hill, C. (2005). Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated with an ability to grow at low pH. *Appl. Environ. Microbiol.* **71,** 2832–2839.

Desvaux, M., and Hebraud, M. (2006). The protein secretion systems in *Listeria*: Inside out bacterial virulence. *FEMS Microbiol. Rev.* **30,** 774–805.

Domann, E., Leimeister-Wachter, M., Goebel, W., and Chakraborty, T. (1991). Molecular cloning, sequencing, and identification of a metalloprotease gene from *Listeria monocytogenes* that is species specific and physically linked to the listeriolysin gene. *Infect. Immun.* **59,** 65–72.

Domann, E., Wehland, J., Rohde, M., Pistor, S., Hartl, M., Goebel, W., Leimeister-Wachter, M., Wuenscher, M., and Chakraborty, T. (1992). A novel bacterial virulence gene in *Listeria monocytogenes* required for host cell microfilament interaction with homology to the proline-rich region of vinculin. *EMBO J.* **11,** 1981–1990.

Domann, E., Zechel, S., Lingnau, A., Hain, T., Darji, A., Nichterlein, T., Wehland, J., and Chakraborty, T. (1997). Identification and characterization of a novel PrfA-regulated gene in *Listeria monocytogenes* whose product, IrpA, is highly homologous to internalin proteins, which contain leucine-rich repeats. *Infect. Immun.* **65,** 101–109.

Dons, L., Eriksson, E., Jin, Y., Rottenberg, M. E., Kristensson, K., Larsen, C. N., Bresciani, J., and Olsen, J. E. (2004). Role of flagellin and the two-component CheA/CheY system of *Listeria monocytogenes* in host cell invasion and virulence. *Infect. Immun.* **72,** 3237–3244.

Dramsi, S., and Cossart, P. (2003). Listeriolysin O-mediated calcium influx potentiates entry of *Listeria monocytogenes* into the human Hep-2 epithelial cell line. *Infect. Immun.* **71,** 3614–3618.

Dramsi, S., Kocks, C., Forestier, C., and Cossart, P. (1993). Internalin-mediated invasion of epithelial cells by *Listeria monocytogenes* is regulated by the bacterial growth state, temperature and the pleiotropic activator prfA. *Mol. Microbiol.* **9,** 931–941.

Dramsi, S., Biswas, I., Maguin, E., Braun, L., Mastroeni, P., and Cossart, P. (1995). Entry of *Listeria monocytogenes* into hepatocytes requires expression of inIB, a surface protein of the internalin multigene family. *Mol. Microbiol.* **16,** 251–261.

Dramsi, S., Bourdichon, F., Cabanes, D., Lecuit, M., Fsihi, H., and Cossart, P. (2004). FbpA, a novel multifunctional *Listeria monocytogenes* virulence factor. *Mol. Microbiol.* **53,** 639–649.

Dussurget, O., Cabanes, D., Dehoux, P., Lecuit, M., Buchrieser, C., Glaser, P., and Cossart, P. (2002). *Listeria monocytogenes* bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. *Mol. Microbiol.* **45,** 1095–1106.

Dussurget, O., Pizarro-Cerda, J., and Cossart, P. (2004). Molecular determinants of *Listeria monocytogenes* virulence. *Annu. Rev. Microbiol.* **58,** 587–610.

Dussurget, O., Dumas, E., Archambaud, C., Chafsey, I., Chambon, C., Hebraud, M., and Cossart, P. (2005). *Listeria monocytogenes* ferritin protects against multiple stresses and is required for virulence. *FEMS Microbiol. Lett.* **250,** 253–261.

Engelbrecht, F., Chun, S. K., Ochs, C., Hess, J., Lottspeich, F., Goebel, W., and Sokolovic, Z. (1996). A new PrfA-regulated gene of *Listeria monocytogenes* encoding a small, secreted protein which belongs to the family of internalins. *Mol. Microbiol.* **21,** 823–837.

Faith, N. G., Kathariou, S., Neudeck, B. L., Luchansky, J. B., and Czuprynski, C. J. (2007). A p60 mutant of *Listeria monocytogenes* is impaired in its ability to cause infection in intragastrically inoculated mice. *Microb. Pathog.* **42,** 237–241.

Gaillard, J. L., Berche, P., and Sansonetti, P. (1986). Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. *Infect. Immun.* **52,** 50–55.

Gaillard, J. L., Berche, P., Mounier, J., Richard, S., and Sansonetti, P. (1987). *In vitro* model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. *Infect. Immun.* **55,** 2822–2829.

Gaillard, J. L., Berche, P., Frehel, C., Gouin, E., and Cossart, P. (1991). Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell* **65,** 1127–1141.

Gaillard, J. L., Jaubert, F., and Berche, P. (1996). The inlAB locus mediates the entry of *Listeria monocytogenes* into hepatocytes *in vivo*. *J. Exp. Med*. **183,** 359–369.

Gaillot, O., Pellegrini, E., Bregenholt, S., Nair, S., and Berche, P. (2000). The ClpP serine protease is essential for the intracellular parasitism and virulence of *Listeria monocytogenes*. *Mol. Microbiol*. **35,** 1286–1294.

Garandeau, C., Reglier-Poupet, H., Dubail, I., Beretti, J. L., Berche, P., and Charbit, A. (2002). The sortase SrtA of *Listeria monocytogenes* is involved in processing of internalin and in virulence. *Infect. Immun*. **70,** 1382–1390.

Garifulin, O., and Boyartchuk, V. (2005). *Listeria monocytogenes* as a probe of immune function. *Brief. Funct. Genomic. Proteomic*. **4,** 258–269.

Garner, M. R., Njaa, B. L., Wiedmann, M., and Boor, K. J. (2006). Sigma B contributes to *Listeria monocytogenes* gastrointestinal infection but not to systemic spread in the guinea pig infection model. *Infect. Immun*. **74,** 876–886.

Gedde, M. M., Higgins, D. E., Tilney, L. G., and Portnoy, D. A. (2000). Role of listeriolysin O in cell-to-cell spread of *Listeria monocytogenes*. *Infect. Immun*. **68,** 999–1003.

Geese, M., Loureiro, J. J., Bear, J. E., Wehland, J., Gertler, F. B., and Sechi, A. S. (2002). Contribution of Ena/VASP proteins to intracellular motility of *Listeria* requires phosphorylation and proline-rich core but not F-actin binding or multimerization. *Mol. Biol. Cell* **13,** 2383–2396.

Geoffroy, C., Raveneau, J., Beretti, J. L., Lecroisey, A., Vazquez-Boland, J. A., Alouf, J. E., and Berche, P. (1991). Purification and characterization of an extracellular 29-kilodalton phospholipase C from *Listeria monocytogenes*. *Infect. Immun*. **59,** 2382–2388.

Girardin, S. E., Boneca, I. G., Carneiro, L. A., Antignac, A., Jehanno, M., Viala, J., Tedin, K., Taha, M. K., Labigne, A., Zahringer, U., Coyle, A. J., DiStefano, P. S., *et al.* (2003a). Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science* **300,** 1584–1587.

Girardin, S. E., Boneca, I. G., Viala, J., Chamaillard, M., Labigne, A., Thomas, G., Philpott, D. J., and Sansonetti, P. J. (2003b). Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J. Biol. Chem*. **278,** 8869–8872.

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H., Brandt, P., Chakraborty, T., Charbit, A., Chetouani, F., *et al.* (2001). Comparative genomics of *Listeria* species. *Science* **294,** 849–852.

Gluck, T., Linde, H. J., Scholmerich, J., Muller-Ladner, U., Fiehn, C., and Bohland, P. (2002). Anti-tumor necrosis factor therapy and *Listeria monocytogenes* infection: Report of two cases. *Arthritis Rheum*. **46,** 2255–2257.

Goldfine, H., Bannam, T., Johnston, N. C., and Zuckert, W. R. (1998). Bacterial phospholipases and intracellular growth: The two distinct phospholipases C of *Listeria monocytogenes*. *Symp. Ser. Soc. Appl. Microbiol*. **27,** 7S–14S.

Gordon, S., and Singer, C. (1986). *Listeria monocytogenes* cholecystitis. *J. Infect. Dis*. **154,** 918–919.

Gouin, E., Gantelet, H., Egile, C., Lasa, I., Ohayon, H., Villiers, V., Gounon, P., Sansonetti, P. J., and Cossart, P. (1999). A comparative study of the actin-based motilities of the pathogenic bacteria *Listeria monocytogenes*, *Shigella flexneri* and *Rickettsia conorii*. *J. Cell Sci*. **112**(Pt 11), 1697–1708.

Gouin, E., Egile, C., Dehoux, P., Villiers, V., Adams, J., Gertler, F., Li, R., and Cossart, P. (2004). The RickA protein of Rickettsia conorii activates the Arp2/3 complex. *Nature* **427,** 457–461.

Gouin, E., Welch, M. D., and Cossart, P. (2005). Actin-based motility of intracellular pathogens. *Curr. Opin. Microbiol*. **8,** 35–45.

Greiffenberg, L., Goebel, W., Kim, K. S., Weiglein, I., Bubert, A., Engelbrecht, F., Stins, M., and Kuhn, M. (1998). Interaction of *Listeria monocytogenes* with human brain microvascular endothelial cells: InlB-dependent invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. *Infect. Immun.* **66,** 5260–5267.

Griffin, A. M., and Robbins, M. L. (1944). The flagellation of *Listeria monocytogenes*. *J. Bacteriol.* **48,** 114–115.

Grundling, A., Gonzalez, M. D., and Higgins, D. E. (2003). Requirement of the *Listeria monocytogenes* broad-range phospholipase PC-PLC during infection of human epithelial cells. *J. Bacteriol.* **185,** 6295–6307.

Grundling, A., Burrack, L. S., Bouwer, H. G., and Higgins, D. E. (2004). *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. *Proc. Natl. Acad. Sci. USA* **101,** 12318–12323.

Hain, T., Steinweg, C., Kuenne, C. T., Billion, A., Ghai, R., Chatterjee, S. S., Domann, E., Karst, U., Goesmann, A., Bekel, T., Bartels, D., Kaiser, O., *et al.* (2006). Whole-genome sequence of *Listeria welshimeri* reveals common steps in genome reduction with *Listeria innocua* as compared to *Listeria monocytogenes*. *J. Bacteriol.* **188,** 7405–7415.

Hain, T., Chatterjee, S. S., Ghai, R., Kuenne, C. T., Billion, A., Steinweg, C., Domann, E., Karst, U., Jansch, L., Wehland, J., Eisenreich, W., Bacher, A., *et al.* (2007). Pathogenomics of *Listeria* spp. *Int. J. Med. Microbiol.* **297,** 541–557.

Hamon, M., Bierne, H., and Cossart, P. (2006). *Listeria monocytogenes*: A multifaceted model. *Nat. Rev. Microbiol.* **4,** 423–434.

Hamon, M. A., Batsche, E., Regnault, B., Tham, T. N., Seveau, S., Muchardt, C., and Cossart, P. (2007). Histone modifications induced by a family of bacterial toxins. *Proc. Natl. Acad. Sci. USA* **104,** 13467–13472.

Hardy, J., Francis, K. P., DeBoer, M., Chu, P., Gibbs, K., and Contag, C. H. (2004). Extracellular replication of *Listeria monocytogenes* in the murine gallbladder. *Science* **303,** 851–853.

Hardy, J., Margolis, J. J., and Contag, C. H. (2006). Induced biliary excretion of *Listeria monocytogenes*. *Infect. Immun.* **74,** 1819–1827.

Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., Eng, J. K., Akira, S., Underhill, D. M., and Aderem, A. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410,** 1099–1103.

Humann, J., Bjordahl, R., Andreasen, K., and Lenz, L. L. (2007). Expression of the p60 autolysin enhances NK cell activation and is required for *Listeria monocytogenes* expansion in IFN-gamma-responsive mice. *J. Immunol.* **178,** 2407–2414.

Inohara, N., Ogura, Y., Fontalba, A., Gutierrez, O., Pons, F., Crespo, J., Fukase, K., Inamura, S., Kusumoto, S., Hashimoto, M., Foster, S. J., Moran, A. P., *et al.* (2003). Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J. Biol. Chem.* **278,** 5509–5512.

Ireton, K., Payrastre, B., Chap, H., Ogawa, W., Sakaue, H., Kasuga, M., and Cossart, P. (1996). A role for phosphoinositide 3-kinase in bacterial invasion. *Science* **274,** 780–782.

Ireton, K., Payrastre, B., and Cossart, P. (1999). The *Listeria monocytogenes* protein InlB is an agonist of mammalian phosphoinositide 3-kinase. *J. Biol. Chem.* **274,** 17025–17032.

Jacquet, C., Doumith, M., Gordon, J. I., Martin, P. M., Cossart, P., and Lecuit, M. (2004). A molecular marker for evaluating the pathogenic potential of foodborne *Listeria monocytogenes*. *J. Infect. Dis.* **189,** 2094–2100.

Jin, B., Newton, S. M., Shao, Y., Jiang, X., Charbit, A., and Klebba, P. E. (2006). Iron acquisition systems for ferric hydroxamates, haemin and haemoglobin in *Listeria monocytogenes*. *Mol. Microbiol.* **59,** 1185–1198.

Johansson, J., and Cossart, P. (2003). RNA-mediated control of virulence gene expression in bacterial pathogens. *Trends Microbiol.* **11,** 280–285.

Johansson, J., Mandin, P., Renzoni, A., Chiaruttini, C., Springer, M., and Cossart, P. (2002). An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. *Cell* **110,** 551–561.

Jonquieres, R., Bierne, H., Fiedler, F., Gounon, P., and Cossart, P. (1999). Interaction between the protein InlB of *Listeria monocytogenes* and lipoteichoic acid: A novel mechanism of protein association at the surface of gram-positive bacteria. *Mol. Microbiol.* **34,** 902–914.

Jonquieres, R., Pizarro-Cerda, J., and Cossart, P. (2001). Synergy between the N- and C-terminal domains of InlB for efficient invasion of non-phagocytic cells by *Listeria monocytogenes*. *Mol. Microbiol.* **42,** 955–965.

Joseph, B., Przybilla, K., Stuhler, C., Schauer, K., Slaghuis, J., Fuchs, T. M., and Goebel, W. (2006). Identification of *Listeria monocytogenes* genes contributing to intracellular replication by expression profiling and mutant screening. *J. Bacteriol.* **188,** 556–568.

Kamisango, K., Saiki, I., Tanio, Y., Okumura, H., Araki, Y., Sekikawa, I., Azuma, I., and Yamamura, Y. (1982). Structures and biological activities of peptidoglycans of *Listeria monocytogenes* and *Propionibacterium acnes*. *J. Biochem.* **92,** 23–33.

Kathariou, S., Metz, P., Hof, H., and Goebel, W. (1987). Tn916-induced mutations in the hemolysin determinant affecting virulence of *Listeria monocytogenes*. *J. Bacteriol.* **169,** 1291–1297.

Kayal, S., and Charbit, A. (2006). Listeriolysin O: A key protein of *Listeria monocytogenes* with multiple functions. *FEMS Microbiol. Rev.* **30,** 514–529.

Kayal, S., Lilienbaum, A., Poyart, C., Memet, S., Israel, A., and Berche, P. (1999). Listeriolysin O-dependent activation of endothelial cells during infection with *Listeria monocytogenes*: Activation of NF-kappa B and upregulation of adhesion molecules and chemokines. *Mol. Microbiol.* **31,** 1709–1722.

Kazmierczak, M. J., Mithoe, S. C., Boor, K. J., and Wiedmann, M. (2003). *Listeria monocytogenes* sigma B regulates stress response and virulence functions. *J. Bacteriol.* **185,** 5722–5734.

Keeney, K. M., Stuckey, J. A., and O'Riordan, M. X. (2007). LplA1-dependent utilization of host lipoyl peptides enables *Listeria* cytosolic growth and virulence. *Mol. Microbiol.* **66,** 758–770.

Khelef, N., Lecuit, M., Bierne, H., and Cossart, P. (2006). Species specificity of the *Listeria monocytogenes* InlB protein. *Cell. Microbiol.* **8,** 457–470.

Kim, H., Boor, K. J., and Marquis, H. (2004). *Listeria monocytogenes* sigmaB contributes to invasion of human intestinal epithelial cells. *Infect. Immun.* **72,** 7374–7378.

Knudsen, G. M., Olsen, J. E., and Dons, L. (2004). Characterization of DegU, a response regulator in *Listeria monocytogenes*, involved in regulation of motility and contributes to virulence. *FEMS Microbiol. Lett.* **240,** 171–179.

Ko, R., and Smith, L. T. (1999). Identification of an ATP-driven, osmoregulated glycine betaine transport system in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* **65,** 4040–4048.

Kocks, C., Gouin, E., Tabouret, M., Berche, P., Ohayon, H., and Cossart, P. (1992). *L. monocytogenes*-induced actin assembly requires the actA gene product, a surface protein. *Cell* **68,** 521–531.

Kocks, C., Marchand, J. B., Gouin, E., d'Hauteville, H., Sansonetti, P. J., Carlier, M. F., and Cossart, P. (1995). The unrelated surface proteins ActA of *Listeria monocytogenes* and IcsA of *Shigella flexneri* are sufficient to confer actin-based motility on *Listeria innocua* and *Escherichia coli* respectively. *Mol. Microbiol.* **18,** 413–423.

Lasa, I., David, V., Gouin, E., Marchand, J. B., and Cossart, P. (1995). The amino-terminal part of ActA is critical for the actin-based motility of *Listeria monocytogenes*; the central proline-rich region acts as a stimulator. *Mol. Microbiol.* **18,** 425–436.

Lasa, I., Gouin, E., Goethals, M., Vancompernolle, K., David, V., Vandekerckhove, J., and Cossart, P. (1997). Identification of two regions in the N-terminal domain of ActA involved in the actin comet tail formation by *Listeria monocytogenes*. *EMBO J.* **16,** 1531–1540.

Laurent, V., Loisel, T. P., Harbeck, B., Wehman, A., Grobe, L., Jockusch, B. M., Wehland, J., Gertler, F. B., and Carlier, M. F. (1999). Role of proteins of the Ena/VASP family in actin-based motility of *Listeria monocytogenes*. *J. Cell Biol.* **144,** 1245–1258.

Lecuit, M. (2005). Understanding how *Listeria monocytogenes* targets and crosses host barriers. *Clin. Microbiol. Infect.* **11,** 430–436.

Lecuit, M. (2007). Human listeriosis and animal models. *Microbes Infect.* **9,** 1216–1225.

Lecuit, M., and Cossart, P. (2002). Genetically-modified-animal models for human infections: The *Listeria* paradigm. *Trends Mol. Med.* **8,** 537–542.

Lecuit, M., Dramsi, S., Gottardi, C., Fedor-Chaiken, M., Gumbiner, B., and Cossart, P. (1999). A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes*. *EMBO J.* **18,** 3956–3963.

Lecuit, M., Hurme, R., Pizarro-Cerda, J., Ohayon, H., Geiger, B., and Cossart, P. (2000). A role for alpha-and beta-catenins in bacterial uptake. *Proc. Natl. Acad. Sci. USA* **97,** 10008–10013.

Lecuit, M., Vandormael-Pournin, S., Lefort, J., Huerre, M., Gounon, P., Dupuy, C., Babinet, C., and Cossart, P. (2001). A transgenic model for listeriosis: Role of internalin in crossing the intestinal barrier. *Science* **292,** 1722–1725.

Lecuit, M., Nelson, D. M., Smith, S. D., Khun, H., Huerre, M., Vacher-Lavenu, M. C., Gordon, J. I., and Cossart, P. (2004). Targeting and crossing of the human maternofetal barrier by *Listeria monocytogenes*: Role of internalin interaction with trophoblast E-cadherin. *Proc. Natl. Acad. Sci. USA* **101,** 6152–6157.

Lee, J. W., and Helmann, J. D. (2006). The PerR transcription factor senses H2O2 by metal-catalysed histidine oxidation. *Nature* **440,** 363–367.

Lee, J. W., and Helmann, J. D. (2007). Functional specialization within the Fur family of metalloregulators. *Biometals* **20,** 485–499.

Leifson, E., and Palen, M. I. (1955). Variations and spontaneous mutations in the genus *Listeria* in respect to flagellation and motility. *J. Bacteriol.* **70,** 233–240.

Leimeister-Wachter, M., Domann, E., and Chakraborty, T. (1991). Detection of a gene encoding a phosphatidylinositol-specific phospholipase C that is co-ordinately expressed with listeriolysin in *Listeria monocytogenes*. *Mol. Microbiol.* **5,** 361–366.

Lenz, L. L., and Portnoy, D. A. (2002). Identification of a second *Listeria* secA gene associated with protein secretion and the rough phenotype. *Mol. Microbiol.* **45,** 1043–1056.

Lenz, L. L., Mohammadi, S., Geissler, A., and Portnoy, D. A. (2003). SecA2-dependent secretion of autolytic enzymes promotes *Listeria monocytogenes* pathogenesis. *Proc. Natl. Acad. Sci. USA* **100,** 12432–12437.

Lingnau, A., Domann, E., Hudel, M., Bock, M., Nichterlein, T., Wehland, J., and Chakraborty, T. (1995). Expression of the *Listeria monocytogenes* EGD inlA and inlB genes, whose products mediate bacterial entry into tissue culture cell lines, by PrfA-dependent and -independent mechanisms. *Infect. Immun.* **63,** 3896–3903.

Loupa, C. V., Kouppar, G., Kosionis, N., Zouberi Koliomichali, M., and Lelekis, M. I. (2007). Biliary tract infection caused by *Listeria monocytogenes*. *Clin. Microbiol. Newsl.* **29,** 6–8.

Luo, Q., Rauch, M., Marr, A. K., Muller-Altrock, S., and Goebel, W. (2004). *In vitro* transcription of the *Listeria monocytogenes* virulence genes inlC and mpl reveals overlapping PrfA-dependent and -independent promoters that are differentially activated by GTP. *Mol. Microbiol.* **52,** 39–52.

Mackaness, G. B. (1962). Cellular resistance to infection. *J. Exp. Med.* **116,** 381–406.
Mandin, P., Fsihi, H., Dussurget, O., Vergassola, M., Milohanic, E., Toledo-Arana, A., Lasa, I., Johansson, J., and Cossart, P. (2005). VirR, a response regulator critical for *Listeria monocytogenes* virulence. *Mol. Microbiol.* **57,** 1367–1380.
Mandin, P., Repoila, F., Vergassola, M., Geissmann, T., and Cossart, P. (2007). Identification of new noncoding RNAs in *Listeria monocytogenes* and prediction of mRNA targets. *Nucleic Acids Res.* **35,** 962–974.
Marino, M., Braun, L., Cossart, P., and Ghosh, P. (2000). A framework for interpreting the leucine-rich repeats of the *Listeria internalins*. *Proc. Natl. Acad. Sci. USA* **97,** 8784–8788.
Marino, M., Banerjee, M., Jonquieres, R., Cossart, P., and Ghosh, P. (2002). GW domains of the *Listeria monocytogenes* invasion protein InlB are SH3-like and mediate binding to host ligands. *EMBO J.* **21,** 5623–5634.
Marino, M., Banerjee, M., Copp, J., Dramsi, S., Chapman, T., van der Geer, P., Cossart, P., and Ghosh, P. (2004). Characterization of the calcium-binding sites of *Listeria monocytogenes* InlB. *Biochem. Biophys. Res. Commun.* **316,** 379–386.
Marquis, H., Doshi, V., and Portnoy, D. A. (1995). The broad-range phospholipase C and a metalloprotease mediate listeriolysin O-independent escape of *Listeria monocytogenes* from a primary vacuole in human epithelial cells. *Infect. Immun.* **63,** 4531–4534.
McGann, P., Wiedmann, M., and Boor, K. J. (2007). The alternative sigma factor sigma B and the virulence gene regulator PrfA both regulate transcription of *Listeria monocytogenes* internalins. *Appl. Environ. Microbiol.* **73,** 2919–2930.
Mengaud, J., Braun-Breton, C., and Cossart, P. (1991). Identification of phosphatidylinositol-specific phospholipase C activity in *Listeria monocytogenes*: A novel type of virulence factor? *Mol. Microbiol.* **5,** 367–372.
Mengaud, J., Ohayon, H., Gounon, P., Mege, R. M., and Cossart, P. (1996). E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. *Cell* **84,** 923–932.
Milohanic, E., Pron, B., Berche, P., and Gaillard, J. L. (2000). Identification of new loci involved in adhesion of *Listeria monocytogenes* to eukaryotic cells. European *Listeria* Genome Consortium. *Microbiology* **146**(Pt 3), 731–739.
Milohanic, E., Jonquieres, R., Cossart, P., Berche, P., and Gaillard, J. L. (2001). The autolysin Ami contributes to the adhesion of *Listeria monocytogenes* to eukaryotic cells via its cell wall anchor. *Mol. Microbiol.* **39,** 1212–1224.
Milohanic, E., Glaser, P., Coppee, J. Y., Frangeul, L., Vega, Y., Vazquez-Boland, J. A., Kunst, F., Cossart, P., and Buchrieser, C. (2003). Transcriptome analysis of *Listeria monocytogenes* identifies three groups of genes differently regulated by PrfA. *Mol. Microbiol.* **47,** 1613–1625.
Milohanic, E., Jonquieres, R., Glaser, P., Dehoux, P., Jacquet, C., Berche, P., Cossart, P., and Gaillard, J. L. (2004). Sequence and binding activity of the autolysin-adhesin Ami from epidemic *Listeria monocytogenes* 4b. *Infect. Immun.* **72,** 4401–4409.
Mohamed, W., Darji, A., Domann, E., Chiancone, E., and Chakraborty, T. (2006). The ferritin-like protein Frm is a target for the humoral immune response to *Listeria monocytogenes* and is required for efficient bacterial survival. *Mol. Genet. Genomics* **275,** 344–353.
Mounier, J., Ryter, A., Coquis-Rondon, M., and Sansonetti, P. J. (1990). Intracellular and cell-to-cell spread of *Listeria monocytogenes* involves interaction with F-actin in the enterocytelike cell line Caco-2. *Infect. Immun.* **58,** 1048–1058.
Nadon, C. A., Bowen, B. M., Wiedmann, M., and Boor, K. J. (2002). Sigma B contributes to PrfA-mediated virulence in *Listeria monocytogenes*. *Infect. Immun.* **70,** 3948–3952.
Nair, S., Frehel, C., Nguyen, L., Escuyer, V., and Berche, P. (1999). ClpE, a novel member of the HSP100 family, is involved in cell division and virulence of *Listeria monocytogenes*. *Mol. Microbiol.* **31,** 185–196.

Nair, S., Milohanic, E., and Berche, P. (2000). ClpC ATPase is required for cell adhesion and invasion of *Listeria monocytogenes*. *Infect. Immun.* **68,** 7061–7068.

Newton, S. M., Klebba, P. E., Raynaud, C., Shao, Y., Jiang, X., Dubail, I., Archer, C., Frehel, C., and Charbit, A. (2005). The svpA-srtB locus of *Listeria monocytogenes*: Fur-mediated iron regulation and effect on virulence. *Mol. Microbiol.* **55,** 927–940.

Niebuhr, K., Ebel, F., Frank, R., Reinhard, M., Domann, E., Carl, U. D., Walter, U., Gertler, F. B., Wehland, J., and Chakraborty, T. (1997). A novel proline-rich motif present in ActA of *Listeria monocytogenes* and cytoskeletal proteins is the ligand for the EVH1 domain, a protein module present in the Ena/VASP family. *EMBO J.* **16,** 5433–5444.

Nightingale, K. K., Windham, K., Martin, K. E., Yeung, M., and Wiedmann, M. (2005). Select *Listeria monocytogenes* subtypes commonly found in foods carry distinct nonsense mutations in inlA, leading to expression of truncated and secreted internalin A, and are associated with a reduced invasion phenotype for human intestinal epithelial cells. *Appl. Environ. Microbiol.* **71,** 8764–8772.

O'Neil, H. S., and Marquis, H. (2006). *Listeria monocytogenes* flagella are used for motility, not as adhesins, to increase host cell invasion. *Infect. Immun.* **74,** 6675–6681.

O'Riordan, M., Moors, M. A., and Portnoy, D. A. (2003). *Listeria* intracellular growth and virulence require host-derived lipoic acid. *Science* **302,** 462–464.

Olier, M., Pierre, F., Rousseaux, S., Lemaitre, J. P., Rousset, A., Piveteau, P., and Guzzo, J. (2003). Expression of truncated Internalin A is involved in impaired internalization of some *Listeria monocytogenes* isolates carried asymptomatically by humans. *Infect. Immun.* **71,** 1217–1224.

Olsen, K. N., Larsen, M. H., Gahan, C. G., Kallipolitis, B., Wolf, X. A., Rea, R., Hill, C., and Ingmer, H. (2005). The Dps-like protein Fri of *Listeria monocytogenes* promotes stress tolerance and intracellular multiplication in macrophage-like cells. *Microbiology* **151,** 925–933.

Ooi, A., Hussain, S., Seyedarabi, A., and Pickersgill, R. W. (2006). Structure of internalin C from *Listeria monocytogenes*. *Acta Crystallogr. D Biol. Crystallogr.* **62,** 1287–1293.

Orndorff, P. E., Hamrick, T. S., Smoak, I. W., and Havell, E. A. (2006). Host and bacterial factors in listeriosis pathogenesis. *Vet. Microbiol.* **114,** 1–15.

Pamer, E. G. (2004). Immune responses to *Listeria monocytogenes*. *Nat. Rev. Immunol.* **4,** 812–823.

Parida, S. K., Domann, E., Rohde, M., Muller, S., Darji, A., Hain, T., Wehland, J., and Chakraborty, T. (1998). Internalin B is essential for adhesion and mediates the invasion of *Listeria monocytogenes* into human endothelial cells. *Mol. Microbiol.* **28,** 81–93.

Peel, M., Donachie, W., and Shaw, A. (1988). Temperature-dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and western blotting. *J. Gen. Microbiol.* **134,** 2171–2178.

Popowska, M., and Markiewicz, Z. (2004). Murein-hydrolyzing activity of flagellin FlaA of *Listeria monocytogenes*. *Pol. J. Microbiol.* **53,** 237–241.

Portnoy, D. A., Jacks, P. S., and Hinrichs, D. J. (1988). Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. *J. Exp. Med.* **167,** 1459–1471.

Pucciarelli, M. G., Calvo, E., Sabet, C., Bierne, H., Cossart, P., and Garcia-del Portillo, F. (2005). Identification of substrates of the *Listeria monocytogenes* sortases A and B by a non-gel proteomic analysis. *Proteomics* **5,** 4808–4817.

Py, B. F., Lipinski, M. M., and Yuan, J. (2007). Autophagy limits *Listeria monocytogenes* intracellular growth in the early phase of primary infection. *Autophagy* **3,** 117–125.

Raffelsbauer, D., Bubert, A., Engelbrecht, F., Scheinpflug, J., Simm, A., Hess, J., Kaufmann, S. H., and Goebel, W. (1998). The gene cluster inlC2DE of *Listeria monocytogenes* contains additional new internalin genes and is important for virulence in mice. *Mol. Gen. Genet.* **260,** 144–158.

Raveneau, J., Geoffroy, C., Beretti, J. L., Gaillard, J. L., Alouf, J. E., and Berche, P. (1992). Reduced virulence of a *Listeria monocytogenes* phospholipase-deficient mutant obtained by transposon insertion into the zinc metalloprotease gene. *Infect. Immun.* **60,** 916–921.

Raynaud, C., and Charbit, A. (2005). Regulation of expression of type I signal peptidases in *Listeria monocytogenes*. *Microbiology* **151,** 3769–3776.

Rea, R. B., Gahan, C. G., and Hill, C. (2004). Disruption of putative regulatory loci in *Listeria monocytogenes* demonstrates a significant role for Fur and PerR in virulence. *Infect. Immun.* **72,** 717–727.

Rea, R. B., Hill, C., and Gahan, C. G. (2005). *Listeria monocytogenes* PerR mutants display a small-colony phenotype, increased sensitivity to hydrogen peroxide, and significantly reduced murine virulence. *Appl. Environ. Microbiol.* **71,** 8314–8322.

Reglier-Poupet, H., Frehel, C., Dubail, I., Beretti, J. L., Berche, P., Charbit, A., and Raynaud, C. (2003a). Maturation of lipoproteins by type II signal peptidase is required for phagosomal escape of *Listeria monocytogenes*. *J. Biol. Chem.* **278,** 49469–49477.

Reglier-Poupet, H., Pellegrini, E., Charbit, A., and Berche, P. (2003b). Identification of LpeA, a PsaA-like membrane protein that promotes cell entry by *Listeria monocytogenes*. *Infect. Immun.* **71,** 474–482.

Rich, K. A., Burkett, C., and Webster, P. (2003). Cytoplasmic bacteria can be targets for autophagy. *Cell. Microbiol.* **5,** 455–468.

Roberts, A. J., and Wiedmann, M. (2003). Pathogen, host and environmental factors contributing to the pathogenesis of listeriosis. *Cell. Mol. Life Sci.* **60,** 904–918.

Rouquette, C., de Chastellier, C., Nair, S., and Berche, P. (1998). The ClpC ATPase of *Listeria monocytogenes* is a general stress protein required for virulence and promoting early bacterial escape from the phagosome of macrophages. *Mol. Microbiol.* **27,** 1235–1245.

Sabet, C., Lecuit, M., Cabanes, D., Cossart, P., and Bierne, H. (2005). LPXTG protein InlJ, a newly identified internalin involved in *Listeria monocytogenes* virulence. *Infect. Immun.* **73,** 6912–6922.

Schirm, M., Schoenhofen, I. C., Logan, S. M., Waldron, K. C., and Thibault, P. (2005). Identification of unusual bacterial glycosylation by tandem mass spectrometry analyses of intact proteins. *Anal. Chem.* **77,** 7774–7782.

Schlech, W. F., 3rd (2000). Foodborne listeriosis. *Clin. Infect. Dis.* **31,** 770–775.

Schluter, D., Domann, E., Buck, C., Hain, T., Hof, H., Chakraborty, T., and Deckert-Schluter, M. (1998). Phosphatidylcholine-specific phospholipase C from *Listeria monocytogenes* is an important virulence factor in murine cerebral listeriosis. *Infect. Immun.* **66,** 5930–5938.

Schmeck, B., Beermann, W., van Laak, V., Zahlten, J., Opitz, B., Witzenrath, M., Hocke, A. C., Chakraborty, T., Kracht, M., Rosseau, S., Suttorp, N., and Hippenstiel, S. (2005). Intracellular bacteria differentially regulated endothelial cytokine release by MAPK-dependent histone modification. *J. Immunol.* **175,** 2843–2850.

Schmid, M. W., Ng, E. Y., Lampidis, R., Emmerth, M., Walcher, M., Kreft, J., Goebel, W., Wagner, M., and Schleifer, K. H. (2005). Evolutionary history of the genus *Listeria* and its virulence genes. *Syst. Appl. Microbiol.* **28,** 1–18.

Schnupf, P., and Portnoy, D. A. (2007). Listeriolysin O: A phagosome-specific lysin. *Microbes Infect.* **9,** 1176–1187.

Schubert, W. D., Gobel, G., Diepholz, M., Darji, A., Kloer, D., Hain, T., Chakraborty, T., Wehland, J., Domann, E., and Heinz, D. W. (2001). Internalins from the human pathogen *Listeria monocytogenes* combine three distinct folds into a contiguous internalin domain. *J. Mol. Biol.* **312,** 783–794.

Seeliger, H. P. R., and Jones, D. (1986). The genus *Listeria*. *In* "Bergey's Manual of Systematic Bacteriology," pp. 1235–1245. Williams and Wilkins, Baltimore.

Seveau, S., Pizarro-Cerda, J., and Cossart, P. (2007). Molecular mechanisms exploited by *Listeria monocytogenes* during host cell invasion. *Microbes Infect.* **9,** 1167–1175.

Shen, A., and Higgins, D. E. (2006). The MogR transcriptional repressor regulates nonhierarchal expression of flagellar motility genes and virulence in *Listeria monocytogenes*. *PLoS Pathog*. **2,** e30.

Shen, Y., Naujokas, M., Park, M., and Ireton, K. (2000). InIB-dependent internalization of *Listeria* is mediated by the Met receptor tyrosine kinase. *Cell* **103,** 501–510.

Shen, A., Kamp, H. D., Grundling, A., and Higgins, D. E. (2006). A bifunctional O-GlcNAc transferase governs flagellar motility through anti-repression. *Genes Dev*. **20,** 3283–3295.

Simon, N., Coulanges, V., Andre, P., and Vidon, D. J. (1995). Utilization of exogenous siderophores and natural catechols by *Listeria monocytogenes*. *Appl. Environ. Microbiol*. **61,** 1643–1645.

Skoble, J., Portnoy, D. A., and Welch, M. D. (2000). Three regions within ActA promote Arp2/3 complex-mediated actin nucleation and *Listeria monocytogenes* motility. *J. Cell Biol*. **150,** 527–538.

Skoble, J., Auerbuch, V., Goley, E. D., Welch, M. D., and Portnoy, D. A. (2001). Pivotal role of VASP in Arp2/3 complex-mediated actin nucleation, actin branch-formation, and *Listeria monocytogenes* motility. *J. Cell Biol*. **155,** 89–100.

Sleator, R. D., Gahan, C. G., Abee, T., and Hill, C. (1999). Identification and disruption of BetL, a secondary glycine betaine transport system linked to the salt tolerance of *Listeria monocytogenes* LO28. *Appl. Environ. Microbiol*. **65,** 2078–2083.

Sleator, R. D., Wouters, J., Gahan, C. G., Abee, T., and Hill, C. (2001). Analysis of the role of OpuC, an osmolyte transport system, in salt tolerance and virulence potential of *Listeria monocytogenes*. *Appl. Environ. Microbiol*. **67,** 2692–2698.

Sleator, R. D., Wemekamp-Kamphuis, H. H., Gahan, C. G., Abee, T., and Hill, C. (2005). A PrfA-regulated bile exclusion system (BilE) is a novel virulence factor in *Listeria monocytogenes*. *Mol. Microbiol*. **55,** 1183–1195.

Smith, G. A., Marquis, H., Jones, S., Johnston, N. C., Portnoy, D. A., and Goldfine, H. (1995). The two distinct phospholipases C of *Listeria monocytogenes* have overlapping roles in escape from a vacuole and cell-to-cell spread. *Infect. Immun*. **63,** 4231–4237.

Sousa, S., Cabanes, D., El-Amraoui, A., Petit, C., Lecuit, M., and Cossart, P. (2004). Unconventional myosin VIIa and vezatin, two proteins crucial for *Listeria* entry into epithelial cells. *J. Cell Sci*. **117,** 2121–2130.

Sousa, S., Cabanes, D., Archambaud, C., Colland, F., Lemichez, E., Popoff, M., Boisson-Dupuis, S., Gouin, E., Lecuit, M., Legrain, P., and Cossart, P. (2005). ARHGAP10 is necessary for alpha-catenin recruitment at adherens junctions and for *Listeria* invasion. *Nat. Cell Biol*. **7,** 954–960.

Sousa, S., Cabanes, D., Bougneres, L., Lecuit, M., Sansonetti, P., Tran-Van-Nhieu, G., and Cossart, P. (2007). Src, cortactin and Arp2/3 complex are required for E-cadherin-mediated internalization of *Listeria* into cells. *Cell. Microbiol*. **9,** 2629–2643.

Stack, H. M., Sleator, R. D., Bowers, M., Hill, C., and Gahan, C. G. (2005). Role for HtrA in stress induction and virulence potential in *Listeria monocytogenes*. *Appl. Environ. Microbiol*. **71,** 4241–4247.

Suarez, M., Gonzalez-Zorn, B., Vega, Y., Chico-Calero, I., and Vazquez-Boland, J. A. (2001). A role for ActA in epithelial cell invasion by *Listeria monocytogenes*. *Cell. Microbiol*. **3,** 853–864.

Sue, D., Boor, K. J., and Wiedmann, M. (2003). Sigma(B)-dependent expression patterns of compatible solute transporter genes opuCA and lmo1421 and the conjugated bile salt hydrolase gene bsh in *Listeria monocytogenes*. *Microbiology* **149,** 3247–3256.

Swaminathan, B., and Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. *Microbes Infect*. **9,** 1236–1243.

Tang, P., Rosenshine, I., Cossart, P., and Finlay, B. B. (1996). Listeriolysin O activates mitogen-activated protein kinase in eucaryotic cells. *Infect. Immun*. **64,** 2359–2361.

Taylor, C. M., Beresford, M., Epton, H. A., Sigee, D. C., Shama, G., Andrew, P. W., and Roberts, I. S. (2002). *Listeria monocytogenes* relA and hpt mutants are impaired in surface-attached growth and virulence. *J. Bacteriol.* **184,** 621–628.

Thedieck, K., Hain, T., Mohamed, W., Tindall, B. J., Nimtz, M., Chakraborty, T., Wehland, J., and Jansch, L. (2006). The MprF protein is required for lysinylation of phospholipids in listerial membranes and confers resistance to cationic antimicrobial peptides (CAMPs) on *Listeria monocytogenes*. *Mol. Microbiol.* **62,** 1325–1339.

Theriot, J. A., Mitchison, T. J., Tilney, L. G., and Portnoy, D. A. (1992). The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization. *Nature* **357,** 257–260.

Tilney, L. G., and Portnoy, D. A. (1989). Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *J. Cell Biol.* **109,** 1597–1608.

Tilney, L. G., Connelly, P. S., and Portnoy, D. A. (1990). Actin filament nucleation by the bacterial pathogen, *Listeria monocytogenes*. *J. Cell Biol.* **111,** 2979–2988.

Vazquez-Boland, J. A., Kocks, C., Dramsi, S., Ohayon, H., Geoffroy, C., Mengaud, J., and Cossart, P. (1992). Nucleotide sequence of the lecithinase operon of *Listeria monocytogenes* and possible role of lecithinase in cell-to-cell spread. *Infect. Immun.* **60,** 219–230.

Vazquez-Boland, J. A., Dominguez-Bernal, G., Gonzalez-Zorn, B., Kreft, J., and Goebel, W. (2001a). Pathogenicity islands and virulence evolution in *Listeria*. *Microbes Infect.* **3,** 571–584.

Vazquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., Gonzalez-Zorn, B., Wehland, J., and Kreft, J. (2001b). *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* **14,** 584–640.

Veiga, E., and Cossart, P. (2005). *Listeria* hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. *Nat. Cell Biol.* **7,** 894–900.

Veiga, E., and Cossart, P. (2006). The role of clathrin-dependent endocytosis in bacterial internalization. *Trends Cell Biol.* **16,** 499–504.

Veiga, E., Guttman, J. A., Bonazzi, M., Boucrot, E., Toledo-Arana, A., Lin, A. E., Enninga, J., Pizarro-Cerda, J., Finlay, B. B., Kirchhausen, T., and Cossart, P. (2007). Invasive and adherent bacterial pathogens co-opt host clathrin for infection. *Cell Host Microbe* **2,** 340–351.

Wadsworth, S. J., and Goldfine, H. (2002). Mobilization of protein kinase C in macrophages induced by *Listeria monocytogenes* affects its internalization and escape from the phagosome. *Infect. Immun.* **70,** 4650–4660.

Way, S. S., Thompson, L. J., Lopes, J. E., Hajjar, A. M., Kollmann, T. R., Freitag, N. E., and Wilson, C. B. (2004). Characterization of flagellin expression and its role in *Listeria monocytogenes* infection and immunity. *Cell. Microbiol.* **6,** 235–242.

Wemekamp-Kamphuis, H. H., Wouters, J. A., Sleator, R. D., Gahan, C. G., Hill, C., and Abee, T. (2002). Multiple deletions of the osmolyte transporters BetL, Gbu, and OpuC of *Listeria monocytogenes* affect virulence and growth at high osmolarity. *Appl. Environ. Microbiol.* **68,** 4710–4716.

Williams, T., Joseph, B., Beier, D., Goebel, W., and Kuhn, M. (2005). Response regulator DegU of *Listeria monocytogenes* regulates the expression of flagella-specific genes. *FEMS Microbiol. Lett.* **252,** 287–298.

Wollert, T., Pasche, B., Rochon, M., Deppenmeier, S., van den Heuvel, J., Gruber, A. D., Heinz, D. W., Lengeling, A., and Schubert, W. D. (2007). Extending the host range of *Listeria monocytogenes* by rational protein design. *Cell* **129,** 891–902.

Yang, Y., Liu, B., Dai, J., Srivastava, P. K., Zammit, D. J., Lefrancois, L., and Li, Z. (2007). Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. *Immunity* **26,** 215–226.