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CHAPTER 1.2.11

Listeria monocytogenes and the Genus *Listeria*

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Introduction

The genus *Listeria* contains six species, two of which are pathogenic: *Listeria monocytogenes*, the food-borne human pathogen responsible for listeriosis, on which this chapter is focused, and *L. ivanovii*, an animal pathogen. Listeriae are Gram-positive rod-shaped bacteria with low G+C content, which are found in a variety of animals and niches, including processed food. They are resistant to extreme conditions, such as low temperature or high salt, demonstrating a great adaptability to their environment (Vazquez-Boland et al., 2001).

Listeria monocytogenes infections cause gastroenteritis, meningitis, neuro-encephalitis, chorioamnionitis, abortions, and neonatal infections. Listeriosis is associated with a high mortality rate, particularly in immunocompromized individuals (Schlech, 2000). In addition to its medical importance, *L. monocytogenes* contamination of food products raises important economic issues in the food industry.

Listeria monocytogenes is a facultative intracellular pathogen. It has evolved a panoply of virulence factors, which exploit important cellular processes during infection (Cossart et al., 2003; Cossart and Sansonetti, 2004). *Listeria monocytogenes* has emerged as a genetically manipulable tool to address key cell biology processes, such as phagocytosis, actin-based motility and signaling through growth factor receptors (Cameron et al., 2000; Cossart and Bierne, 2001; Bierne and Cossart, 2002a). Moreover, as a result of its intracellular life, *L. monocytogenes* mediates a strong T-cell response and is widely used as a model to study the CD8-mediated immunity of intracellular parasites (Edelson and Unanue, 2000; Badovinac et al., 2003; Lara-Tejero and Pamer, 2004). The detailed mechanisms of the immune response to *L. monocytogenes* will not be treated in this review, as they are elegantly and largely reviewed (Edelson and Unanue, 2000; Unanue, 2002; Badovinac et al., 2003; Lara-Tejero and Pamer, 2004, Pamer, 2004). The combined use of genetics, cell biology, functional genomics and post-sequencing studies has led to a precise understanding of *L. monocytogenes* infections. Comparative genomics of *L. monocytogenes* with the nonpathogenic species *L. innocua*, and other *Listeria* species can now be fully exploited for the study of virulence, regulation and biodiversity of Listeriae (Glaser et al., 2001; Cabanes et al., 2002; Buchrieser et al., 2003; Doumith et al., 2004a).

The Discovery of Listeriosis and Listeriae

Listeriosis was first described in the late 1920s and proposed to be contracted through oral contamination. This hypothesis was confirmed in the 1960s. A series of outbreaks in industrialized countries during the 1970s and 1980s definitely established that *L. monocytogenes* was indeed responsible for food-borne listeriosis (Schlech, 1984; Rocourt and Cossart, 1997).

The first official strain of the human pathogen *L. monocytogenes* was isolated in 1924 after an animal carehouse outbreak among rabbits and guinea pigs exhibiting severe mononucleosis (Murray et al., 1926). A clinical isolate of *L. monocytogenes* from a case of human meningitis was deposited at the Institut Pasteur in 1921 (Dumont and Cotoni, 1921). The first strain of the animal pathogen, *L. ivanovii*, was identified in the 1960s from lambs with congenital listeriosis (Ivanov, 1962).

Classification

Listeria spp. are small Gram-positive, nonspore forming, noncapsulated bacilli, generally motile at low temperatures (20°C), although some *Listeria* strains are nonmotile at 37°C because of lack of flagellin expression at this temperature (Way et al., 2004). Their DNA is characterized by a low G+C content (36–42%). They are related to *Clostridium*, *Bacillus*, *Enterococcus*, *Streptococcus* and *Staphylococcus* (Seeliger and Jones, 1986; Schmid et al., 2005). On the basis of DNA-

DNA hybridization, multi-locus enzyme electrophoresis (MEE), rRNA gene restriction patterns, and 16S rRNA sequencing, the genus *Listeria* comprises six species and two subspecies: *L. monocytogenes*, *L. ivanovii* subsp. *ivanovii*, *L. ivanovii* subsp. *londoniensis*, *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. grayi*. *Listeria monocytogenes* is a human and animal pathogen, and *L. ivanovii* is pathogenic for animals, mainly sheep and cattle. On rare occasions, *L. ivanovii* and *L. seeligeri* were shown to be associated with human infections (Rocourt and Cossart, 1997; Schmid et al., 2005).

Fig. 1. *Listeria* spp. grown on RAPID'L. MONO medium. *Listeria monocytogenes* appears as blue colonies while other *Listeria* spp. appear as white colonies. Reproduced with permission from BIO-RAD, Marnes La Coquette, France.

Isolation and Characterization

Reservoirs

Listeriae are found in a wide variety of reservoirs ranging from soil, rotting plants, water, cattle milk and food to numerous animal species and humans. There is an asymptomatic carriage of *L. monocytogenes* in the intestinal tract (Seeliger and Jones, 1986; Grif et al., 2001). Listeriae can also colonize various inert surfaces and can form biofilms on food-processing surfaces (Roberts and Wiedman, 2003).

Detection and Isolation

Isolation of *Listeria* from contaminated or infected material can be obtained by direct plating on tryptic soy base agar supplemented with blood for human samples, but an enrichment step followed by plating on selective media is recommended for polycontaminated samples, such as food products. Enrichment procedures vary but are generally based on a one- or two-step enrichment in liquid selective broth, followed by plating on selective solid media, which allow identification of Listeriae (LPM, Oxford, or PALCAM agar), or specific identification of *L. monocytogenes* strains (Rapid L. Mono, ALOA [Agar *Listeria* Ottavani and Agosti], and Chromagar) (Figs. 1 and 2). Rapid detection of Listeriae in food isolates, for example, can be performed using immunoassay-based kits. However, PCR (polymerase chain reaction) is the technique of choice for rapid detection of *L. monocytogenes* in both clinical and food isolates. Isolation remains indispensable for epidemiological studies (Swaminathan et al., 1995; Allerberger, 2003).

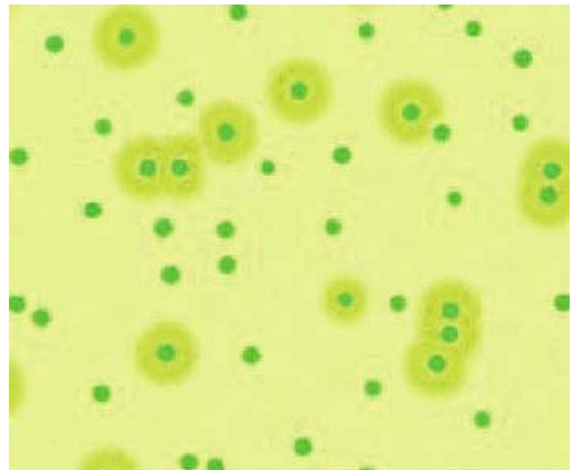

Fig. 2. *Listeria* spp. grown on ALOA (Agar *Listeria* Ottavani and Agosti) chromogenic medium. *Listeria monocytogenes* colonies are surrounded by a halo while other *Listeria* spp. are not. Reproduced with permission from AES Laboratoire, Ker Lann, France.

Identification

Genus identification is based on classical tests including Gram staining, observation of motility, and biochemical reactions, such as catalase and acid production from D-glucose (Swaminathan et al., 1995). Identification of *Listeria* spp. is based on a few biochemical markers and on hemolysis (Table 1). *Listeria* spp. can be identified using an API-*Listeria*® test, a set of 10 biochemical tests, including arylamidase, which discriminates pathogenic *L. monocytogenes* from nonpathogenic *L. innocua* (Swaminathan et al., 1995) (Fig. 3). Hemolysis is produced by *L. monocytogenes* but also by *L. ivanovii* and *L. seeligeri*. Hemolysis of *L. monocytogenes* is narrow and does not develop beyond the edge of colonies. It is due to listeriolysin O (LLO), a β-hemolysin known to be a major virulence factor. Hemolysis of *L. ivanovii* is wider, often bizonal, with a first ring of complete hemolysis and a

Table 1. Biochemical properties of *Listeria* species.

Property	*L. monocytogenes*	*L. ivanovii*	*L. seeligeri*	*L. innocua*	*L. welshimeri*	*L. grayi*
β-Hemolysis	+	+	+	–	–	–
CAMP reaction						
S. aureus	+	–	+	–	–	–
R. equi	–	+	–	–	–	–
Acid production from						
Mannitol	–	–	–	–	–	+
α-Methyl-D-mannoside	+	–	–	+	+	+
L-Rhamnose	+	–	–	V	V	–
Soluble starch	–	–	ND	–	ND	+
D-Xylose	–	+	+	–	+	–
Ribose	–	V	–	–	–	+
Hippurate hydrolysis	+	+	ND	+	ND	–
Reduction of nitrate	–	–	–	–	–	+

Symbols and abbreviations: +, present; –, absent; CAMP, Christie. Atkins, Munch-Peterson; V, variable; and ND, not determined.
From Swaminathan et al. (1995).

Fig. 3. The API-*Listeria*® test. Discrimination between *L. monocytogenes* (upper panel) and *L. innocua* (lower panel). From Allerberger et al. (2003), with permission.

second ring of incomplete hemolysis. *Listeria seeligeri* is weakly hemolytic (Fig. 4). Hemolysis of *L. ivanovii* and *L. seeligeri* is due to listeriolysin homologues, ivanolysin O and seeligerolysin O, respectively, and for *L. ivanovii* to the sphingomyelinase SmcL (Leimeister-Wachter and Chakraborty, 1989; Vazquez-Boland et al., 1989; Haas et al., 1992; Gonzalez-Zorn et al., 1999). The natural hemolysis of Listeriae is specifically enhanced by the products released by other hemolytic bacteria, *Staphylococcus aureus* for *L. monocytogenes* and *L. seeligeri*, or *Rhodococcus equi* for *L. ivanovii* in the CAMP test (Christie, Atkins, Munch-Petersen). In this assay, the streaking of Listeriae and *S. aureus* or *Rhodococcus equi* in close proximity and perpendicularly gives rise to a characteristic shovel-shaped patch of synergistic hemolysis (Swaminathan et al., 1995). In *L. ivanovii*, the bizonal hemolysis and the effect observed in the CAMP test are due to the SmcL phospholipase (Gonzalez-Zorn et al., 1999) (Fig. 5).

Other approaches, such as growth on chromogenic selective media, Rapid L-mono, or ALOA, allow identification of *L. monocytogenes*. Identification on Rapid L-mono plates is based on chromogenic detection of phosphatidylinositol (PI) phospholipase C (PLC-A) and on xylose fermentation differentially produced by *Listeria* species. *Listeria monocytogenes* appears as blue colonies (phospholipase C [PLC] positive) without halo (xylose negative), while *L. ivanovii* appears as blue colonies surrounded by a yellow halo (PLC and xylose positive). Other *Listeria* species appear as white colonies. In addition, chemiluminescent DNA probe assays allow rapid confirmation of *L. monocytogenes* from primary isolation colonies. Finally, sequencing of the 16S rRNA, ribotyping, or pulsed-field gel electrophoresis (PFGE), can be used as an identification method for species determination or subdivision within species (Brosch et al., 1996; Allerberger, 2003).

Typing

Although different methods are available to characterize isolates *L. monocytogenes* [phage typing,

ribotyping, DNA macro-restriction analysis, random amplified polymorphic DNA (RAPD) and multi-locus enzyme electrophoresis (MEE)], serotyping and DNA macro-restriction analysis are methods of choice for outbreak investigations. The 16 serovars of *Listeria* are based on the expression of somatic (O) and flagellar (H) antigens (Table 2), with 13 *L. monocytogenes* serovars that can cause disease. Approximately 95% of the human isolates belong to serovars 4b, 1/2a, and 1/2b. Strikingly, serovars 4b are found in most of the invasive foodborne outbreaks worldwide and in up to 50% of the sporadic cases of listeriosis, while serogroups 1/2 (including 1/2a, 1/2b and 1/2c) are mostly associated with sporadic cases (Rocourt and Cossart, 1997). More recently, multiplex PCR was proposed as an alternative to serotyping (Doumith et al., 2004b). The method allows to differentiate the major serovars, 1/2a, 1/2b, 1/2c and 4b, of *L. monocytogenes*.

Techniques based on phenotypic differentiation (phage typing and bacteriocinotyping) or on molecular characterization (MEE and DNA typing methods) were developed to further characterize strains of the same serovar. Phage typing is based on the testing of the isolates for their sensitivity to a set of phages isolated from lysogenic *Listeria* strains. MEE allows the determination of a pattern of enzyme activities that are specific for *Listeria* subtypes. Both techniques have been successfully applied for epidemiological studies. Molecular methods, such as characterization of chromosomal DNA by restriction endonuclease analysis, ribotyping, RAPD, or PFGE are also used to type *Listeria* strains. PFGE is the most discriminatory method to identify *L. monocytogenes* and is recommended, together

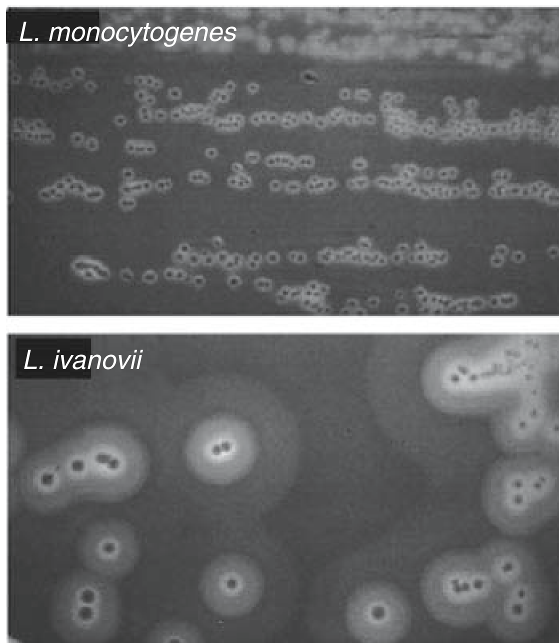

Fig. 4. Hemolysis on blood agar plates after 24 h. *Listeria monocytogenes* produces a narrow zone of hemolysis and *L. ivanovii* produced a wider bizonal hemolysis. From Vazquez-Boland et al. (2001), with permission.

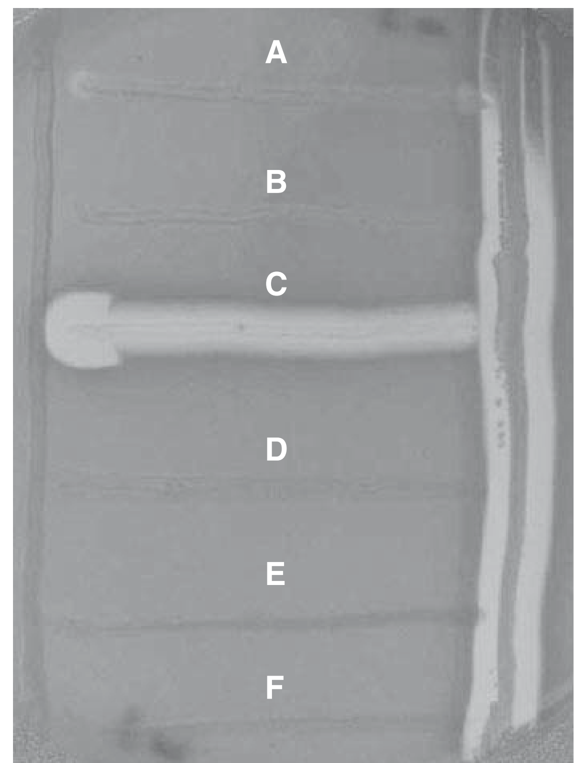

Fig. 5. The CAMP (Christie, Atkins, Munch-Peterson) test. Blood agar plates streaked horizontally with *L. monocytogenes* (A), *L. seeligeri* (B), *L. ivanovii* (C), *L. innocua* (D), *L. grayi* (E) and *L. welshimeri* (F) or vertically with *Rhodococcus equi* (left) or *Staphylococcus aureus* (right). Adapted from Allerberger et al. (2003), with permission.

Table 2. Serovars of the genus *Listeria*.

L. monocytogenes	*L. ivanovii*	*L. seeligeri*	*L. innocua*	*L. welshimeri*	*L. grayi*
1/2a, 1/2b, and 1/2c	5	1/2a, 1/2b, and 1/2c		1/2b	
3a, 3b, and 3c					
4a, 4ab, 4b, 4c, 4d, and 4e		4b and 4d	4ab	4c	
		6b	6a and 6b	6a and 6b	
7		US*	US	US	S

Abbreviations: US, undesignated serovar; S, specific; and * is both US and S.
From Swaminathan et al. (1995).

with serotyping, for epidemiological surveillance (Swaminathan et al., 1995; Brosch et al., 1996).

Serodiagnosis and Determination of Pathogenicity

Serologic tests cannot be used directly for diagnosis because of an antigenic cross-reactivity between *L. monocytogenes* and other Gram-positive bacteria (Swaminathan et al., 1995). However, detection of anti-listeriolysin antibodies, after adsorption on streptolysin O of *Streptococcus pyogenes*, is a good indicator of infection (Berche et al., 1990). Determination of the pathogenicity of Listeriae in animal models is not used routinely for identification (Swaminathan et al., 1995).

Physiology

Growth Conditions: pH, Temperature and Salts

Listeriae are facultative anaerobic bacteria, a property that might be advantageous during infection. They grow perfectly well in laboratory aerobic conditions. Although BHI (brain heart infusion) is the medium of choice for growth in vitro, Listeriae can grow in several other media such as Luria broth (LB). A minimal medium previously used (Premaratne et al., 1991) was recently readapted based on the analysis of the genome sequence of *L. monocytogenes* (Tsai and Hodgson, 2003). One of the key properties of *L. monocytogenes* resides in its ability to grow in a wide range of temperatures (1–45°C), which favors resistance and enrichment in foods. However, optimal growth occurs at 30–37°C. Listeriae are killed at 60°C, making pasteurization a good technique to eliminate the bacteria from dairy products (Seeliger and Jones, 1986). Analysis of *L. monocytogenes* grown at different temperatures and of cold-sensitive mutants revealed that temperature adaptation involves modifications of the fatty acid composition of the bacterial membrane (Annous et al., 1997). Listeriae also resist relatively extreme pH and salt conditions (pH 4.5–9 and 10% NaCl), but optimal growth occurs at neutral pH and 0.5% NaCl (McClure et al., 1991). The striking resistance of Listeriae to harsh external conditions accounts for its wide distribution in multiple habitats and explains the increasing numbers of food-borne outbreaks in industrialized countries.

Nutrients

Carbohydrates, amino acids (cysteine, glutamine, isoleucine, leucine and valine) and vitamins (biotin, riboflavin, thiamine and thioctic acid) are required for *Listeria* growth (Seeliger and Jones, 1986). An extensive study of the behavior of several auxotrophic mutants revealed that threonine, adenine or phenylalanine–tryptophan-tyrosine auxotrophs were less virulent (Marquis et al., 1993). Iron and some amino acids (arginine, histidine, methionine and tryptophan) stimulate *L. monocytogenes* growth. Activated charcoal or cellobiose have no effect on growth but modulate the expression of virulence genes (Cowart and Fosters, 1985; Seeliger and Jones, 1986; Park and Kroll, 1993; Ripio et al., 1996; Huillet et al., 1999).

Inhibitory Agents and Antibiotics

Virulent *Listeria* strains are resistant to bile (up to 40%) on agar plates. This is partly due to the expression of a bile salt hydrolase, encoded by the *bsh* gene (Dussurget et al., 2002) and other bile tolerance genes encoded by the locus *btlA* (Begley et al., 2003). Disinfectants are active on Listeriae allowing efficient treatment of surfaces in contact with food, but serum and milk reduce their bactericidal activities, emphasizing the attention drawn to hygiene in food industries (Best et al., 1990).

While antibiotic sensitivity might vary between strains, most Listeriae are sensitive to many antibiotics, such as aminoglycosides, tetracyclines, macrolides, chloramphenicol and penicillins. Some of them are used to treat listeriosis, e.g., usually ampicillin in combination with an aminoglycoside (Charpentier et al., 1995; Hof et al., 1997; Troxler et al., 2000). *Listeria* strains are naturally resistant to antibiotics such as cephalosporins or sulfonamides. Resistance to nalidixic acid is exploited to enrich samples in *L. monocytogenes*, and most enrichment media contain this antibiotic. Although antibiotic resistance of Listeriae is not yet a concern for clinics, new tetracycline- or trimethoprim-resistant strains have recently emerged (Poyart-Salmeron et al., 1990; Poyart-Salmeron et al., 1992; Charpentier et al., 1995; Charpentier and Courvalin, 1999).

The Human Disease: Epidemiological and Clinical Aspects

Epidemiology

Listeria monocytogenes had been isolated in humans as early as the 1920s (Murray et al., 1926; Pirie, 1927). Nevertheless, it was not identified as an important cause of neonatal infection until after World War II in Germany (Potel, 1952). The

development and use of immunosuppressive agents in the second half of the twentieth century led to the identification of listeriosis cases in immunocompromized patients (Louria et al., 1967). Subsequently, the development of highly immunosuppressive therapies for organ or bone marrow transplantations led to a great expansion of the immunocompromized population and the subsequent identification of immunosuppression as a major risk factor for listeriosis (Stamm et al., 1982). More recently, the human immunodeficiency virus (HIV) epidemic has markedly enlarged the immunodeficient population, with a relative risk of developing listeriosis 500 times higher in acquired immunodeficiency syndrome (AIDS) patients as compared to the general population (Jurado et al., 1993). Globally, human listeriosis remains a rare disease, and its prevalence is declining in industrialized countries in which food control measures have been implemented. The attack rate is considered to be around 7 per million, leading to 2500 cases and around 500 deaths per year in the United States (Gellin et al., 1991; Lorber, 1997; Vazquez-Boland et al., 2001; Wing and Gregory, 2002). Infection is more common in children (100 cases per million) and elderly (14 cases per million). Pregnant women are 20 times more likely to develop listeriosis than the general population (Broome, 1993).

It has been observed very early in ruminants that oral ingestion of *L. monocytogenes* was associated with listeriosis (Low and Renton, 1985). This led to the hypothesis that *L. monocytogenes* was also a human foodborne pathogen. However, not until the 1980s was the validity of this hypothesis formally demonstrated, and it has now been widely confirmed by other investigators (Schlech et al., 1983). A number of listeriosis outbreaks and sporadic cases consequent to the ingestion of contaminated food products have been reported, as well as the origin of contamination (Table 3). Indeed, vegetables can become contaminated from the soil or from manure used as fertilizer, and animals can carry the bacterium asymptomatically and contaminate foods of animal origin, such as meats and dairy products. *Listeria monocytogenes* is found in a variety of raw foods, such as uncooked meats and vegetables, or processed foods that become contaminated after processing, such as soft cheeses and cold cuts at deli counters (Fleming et al., 1985; Linnan et al., 1988; Schwartz et al., 1989; Riedo et al., 1994; Bula et al., 1995; Salamina et al., 1996; Dalton et al., 1997; Goulet et al., 1998; Aureli et al., 2000; Ooi et al., 2005). Unpasteurized milk or foods made from unpasteurized milk may contain the bacterium. *Listeria monocytogenes* is killed by pasteurization and cooking. However, in certain ready-to-eat foods such as hot dogs and deli meats, contamination may occur after cooking before packaging. Government agencies and the food industry have taken steps to reduce contamination of food by *L. monocytogenes*. The United States Food and Drug Administration (FDA) (http://www.fda.gov/) and the United States Department of Agriculture (USDA) (http://www.usda.gov) monitor food regularly. When a processed food is found to be contaminated, food monitoring and plant inspection are intensified, and if necessary, the implicated food is recalled (for details, see the web sites of the World Health Organization (http://www.who.int/foodsafety/en/), Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov), (http://www.foodsafety.gov/~fsg/fsgprobs.html{Gateway to Government Food Safety Information} or http://www.afssa. fr{Agence française de sécurité sanitaire des aliments}).

Most human foodborne infections are associated with high prevalence but low morbi-mortality. The situation is different for human listeriosis, which is a rare but very serious infec-

Table 3. Main listeriosis outbreaks in Europe and the United States.

Year	Location	Number of cases	Perinatal cases (%)	Mortality rate (%)	Source of contamination
1980–1981	Canada	41	83	34	Cole slaw
1983	New England, USA	49	14	29	Pasteurized milk
1983–1984	Switzerland	57	9	32	Soft cheese
1985	California, USA	142	65	34	Mexican cheese
1986–1987	Pennsylvania, USA	36	11	44	Unknown
1989	Connecticut, USA	10	20	10	Shrimps
1992	France	38	82	32	Deli meat (rillettes)[a]
1993	Italy	39	Unknown	Unknown	Rice salad
1994	Illinois, USA	45	Unknown	Unknown	Chocolate milk
1997	Italy	1566	Unknown	Unknown	Corn salad
1998–1999	United States	1001	Unknown	Unknown	Hot dogs
1999	France	32	12	21	Pork deli meat
2002	Illinois, USA	43	28	31	Turkey deli meat

[a]Rilettes, a spread similar to paté, usually made of pork.

tion, associated with a mortality of up to 30%, even when an adequate treatment is administered. This accounts for the high economic impact associated with listeriosis despite its relative low prevalence (Table 4). Moreover, listeriosis remains under-diagnosed, particularly at its early stages, and this leads to delay in the administration of antimicrobial therapy, which is absolutely critical for a favorable outcome, contrary to most other foodborne infections (Lorber, 1997; Wing and Gregory, 2002). More detailed informations concerning epidemiology can be found at http://www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis_t.htm; http://www.who.int/foodsafety/micro/jemra/assessment/listeria/en/; http://www.invs.sante.fr/beh/2004/09/index.htm.

Clinical Manifestations

Listeria monocytogenes infects humans through the ingestion of contaminated food products. The bacteria can cross the intestinal barrier and disseminate from the mesenteric lymph nodes to the spleen and the liver, from which they may reach the brain or the placenta, resulting in meningitis or encephalitis in immunocompromized patients, abortions in pregnant women, or generalized infections in infected neonates (*granulomatosis infantiseptica*) (Fig. 6). If not controlled properly by the immune system, *L. monocytogenes* infection may also cause septicemia. Highly contaminated food products also infect healthy individuals, resulting mainly in gastroenteritis (Aureli et al., 2000; Vazquez-Boland et al., 2001; Wing and Gregory, 2002; Ooi et al., 2005).

Digestive Manifestations Studies reported by the CDC have shown that 11% of the food samples collected in the framework of food monitoring programs were contaminated with *L. monocytogenes* and that *L. monocytogenes* grew from at least one refrigerator sample of 64% of patients with listeriosis (Pinner et al., 1992). For long, this first step of the infectious process—consumption of *L. monocytogenes*-contaminated food—was considered to be almost always clinically silent. However, it was clearly demonstrated that it may lead to the development of a genuine gastroenteritis (with digestive signs such as nausea, aqueous or bloody diarrhea, abdominal pain, and fever) with an attack rate of up to 70%, particularly in cases of high inocula (Dalton et al., 1997; Schlech, 1997; Aureli et al., 2000). Although a number of *L. monocytogenes* associated gastroenteritis outbreaks have been reported, cases of sporadic enteritis appear to be relatively rare (Schlech et al., 2005). The importance of the inoculum size in the onset of these early clinical signs is supported by the results of oral

Table 4. Cost of foodborne infections in the United States in 1992.

Bacteria	Number of cases in the United States in 1992	Global cost ($US)	Cost per patient ($US)
Salmonella	1,920,000	1,388,000,000	723
Campylobacter jejuni	2,100,000	961,500,000	458
Escherichia coli O157H7	14,058	388,000,000	27,600
Listeria monocytogenes	1,550	221,000,000	142,581

For detailed information about the economics of foodborne diseases, see USDA listing of foodborne diseases.

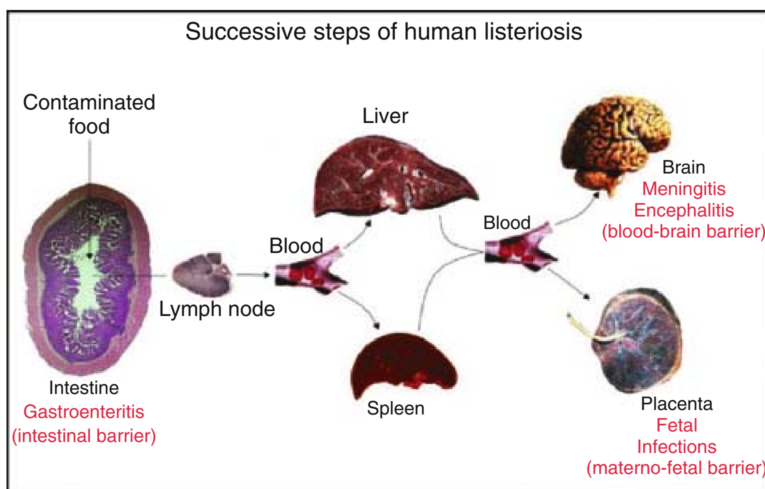

Fig. 6. Successive steps of listeriosis. Organs affected by the infection, symptoms and epithelial barriers crossed by *L. monocytogenes*.

challenges in healthy nonhuman primates, in whom the ingested inoculum had to be higher than 109 organisms to produce a detectable clinical effect (Farber et al., 1991b). Whether these digestive clinical signs mostly account for a toxic effect of *L. monocytogenes* on the intestinal tissue or for the crossing and invasion of the mucosal barrier by *L. monocytogenes* is not known. These digestive manifestations are usually self-limited and resolve spontaneously.

Bacteremia In the population at risk for invasive listeriosis, signs accounting for generalized infection most frequently occur after an incubation period that can be very long (10–70 days). Hematogenous seeding of the pathogen resulting in *L. monocytogenes* bacteremia might be responsible for an influenza-like febrile illness, accompanied by myalgias, arthralgias, headache and backache but might also be clinically silent (Goulet and Marchetti, 1996).

Pregnancy-Associated Listeriosis During pregnancy, particularly the third trimester when pregnancy-associated immunosuppression is the most intense, benign common cold symptoms may be due to *L. monocytogenes*-associated bacteremia and should be treated *a priori* as listeriosis because of the seriousness of possible obstetrical and neonatalogical complications. The specific complications of listeriosis during pregnancy lie in the ability of *L. monocytogenes* to cross the maternofetal barrier and result in placental abscesses, chorioamnionitis, and finally infection of the fetus. This infection is responsible for premature labor, stillbirth, abortion, and neonatal infection, with high mortality. Disseminated infection to the fetus is called granulomatosis infantiseptica and is characterized by the widespread presence of microabscesses and granulomas in liver, spleen and skin. The highest concentrations of *L. monocytogenes* being encountered in the gut and in the lung, infection might be amplified through ingestion of contaminated amniotic fluid rather than solely as a consequence of the hematogenous transplacental route (Lorber, 1997; Schlech, 2000). In cases of neonatal contamination during parturition, a primary septicemic syndrome similar to that associated with *Streptococcus agalactiae* infection occurs sometimes with purulent conjunctivitis and disseminated papular rash evocative of neonatal listeriosis and late-onset meningitis. *Listeria monocytogenes* meningitis is one of the three major causes of meningitis in neonates (Lorber, 1997; Dawson et al., 1999; Schlech, 2000; Lecuit and Cossart, 2001a).

Infection of the Central Nervous System *Listeria monocytogenes* has, in addition to its ability to cross initially the intestinal barrier and the maternofetal barrier in pregnant women, the capacity to cross the blood-brain barrier and reach the central nervous system (CNS). Interestingly, it has been proposed, based on animal studies, that infected bone marrow myeloid cells may promote *Listeria* invasion of the CNS (Join-Lambert et al., 2005). *L. monocytogenes* capacity to cause both acute meningitis and parenchymal brain infection differentiates it from other bacteria frequently responsible for meningitis such as *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae*. Among all bacterial meningitides, *L. monocytogenes* meningitis has the highest mortality rate (22%). *L. monocytogenes* meningitis accounts for 11% of all bacterial meningitis and was the second most common cause of meningitis after *Streptococcus pneumoniae* meningitis in patients older than 50 years (Lecuit and Cossart, 2001a). The clinical features of *L. monocytogenes* meningitis differ from those of other bacterial meningitides in that *L. monocytogenes* meningitis may have a subacute course, and be associated with abnormal movements, seizures, and alteration of consciousness. The onset of such signs is evocative of dissemination to the brain parenchyma. Two different types of dissemination to the brain can occur: encephalitis or intraparenchymal brain abscesses. Encephalitis usually involves particularly the rhombencephalon and clinically appears as a meningitic syndrome that is complicated with cranial nerve deficits, a cerebellar syndrome, or central motor and sensitivity deficits. In two-thirds of the cases, blood cultures are positive. Cerebrospinal fluid culture is positive in half of the cases. The onset of rhombencephalitis is not confined to the immunocompromized host and may also be reported in otherwise healthy adults (Armstrong and Fung, 1993). In a small proportion of *L. monocytogenes* CNS infections (10% with half of them in immunocompromized patients), macroscopic brain abscesses are observed. *Listeria monocytogenes* brain abscesses are preferentially located in subcortical areas, thalamus, pons or medulla. These unusual locations are evocative of their listerial origin (Lorber, 1997; Schlech, 2000). Both encephalitis and parenchymal abscesses are associated with high neurological sequelae and mortality rates.

Localized Infections Rare cases of *L. monocytogenes* endocarditis have been reported, accounting for less than 10% of all *L. monocytogenes* infections and occurring in patients at risk for endocarditis. Rare localized infections in patients at risk for listeriosis have also been reported and include infections due to direct inoculation (conjunctivitis, skin infection or

lymphadenitis), digestive involvement (peritonitis or cholecystis), or hematogenous seeding (abscesses of liver and spleen, pleuropulmonary infections, joint infection and osteomyelitis, pericarditis, myocarditis, arteritis or endophtalmitis) (Lorber, 1997; Schlech, 2000; Lecuit and Cossart, 2001a).

Genetics of *Listeria*

Genetic Elements of *Listeria*

PHAGES Lysogenic bacteriophages have been isolated from *Listeria* species. They belong to the Siphoviridae and Myroviridae families and contain double-stranded DNA of about 40 kb. They do not appear to be involved in virulence, but some have been used to classify *Listeria* strains by phage typing (Cossart and Mengaud, 1989a; McLauchlin et al., 1996). Phages of *Listeria monocytogenes* are, with a few exceptions, specific with respect to the serogroup of the host cells. The major serogroups (1/2 and 4) can be differentiated on the basis of somatic antigen composition and sugar substitution of cell wall teichoic acids (TAs) (Fiedler and Ruhland, 1987). These carbohydrates also serve as a primary receptor for the serogroup specific phages (Wendlinger et al., 1996). In 2000, the complete nucleotide sequence and structural analysis of the serogroup 1/2-specific bacteriophage A118 were reported (Loessner et al., 2000). The A118 genome is a 40.8-kb, linear, circularly permuted, terminally redundant collection of double-stranded DNA molecules. Site-specific integration of the A118 prophage occurs into a gene homologous to *comK* from *Bacillus subtilis*, resulting probably in the inactivation of this gene. Comparative analysis of the A118 genome structure with other bacteriophages revealed local, but sometimes extensive similarities with a number of phages spanning a broader phylogenetic range of low G+C bacteria, implying relatively recent exchange of genes and genetic modules (Loessner et al., 2000). The genome and the proteome of a serogroup-4-specific phage, PSA, were described in 2003 (Zimmer et al., 2003). PSA is a temperate phage isolated from *L. monocytogenes* strain Scott A, a clinical epidemic strain. The genome of the bacteriophage PSA is 37.6 kb long and contains 57 open-reading frames, which are organized into three major transcriptional units. The PSA integration site is *attB* located at the 3′ end of the single-copy $tRNA^{Arg}$ gene, where phage nucleotides reconstitute its function (Lauer et al., 2002). Bioinformatics revealed only few similarities of PSA with the *Listeria* phage A118. The analyses also revealed the first case of a +1 frameshifting among dsDNA phages, and the utilization of a 3′ pseudoknot to stimulate such an event (Zimmer et al., 2003). On the basis of the analyses of these two bacteriophages and the identification of their respective integration sites, two site-specific integration vectors, pPL1 and pPL2, which utilize the listeriophage U153 integrase, were constructed (Lauer et al., 2002) (Fig. 7). pPL1 integrates in the *comK* attachment site and pPL2 uses the PSA attachment site in the $tRNA^{Arg}$ gene. These plasmids were used in *L. monocytogenes* enabling complementation experiments (Lauer et al., 2002).

PLASMIDS Many strains of *Listeria* species contain one large cryptic plasmid. Several plasmids of various sizes were detected in *Listeria*, but hybridization and restriction analysis revealed a high degree of homology between them (Kolstad et al., 1991). In certain cases, *L. monocytogenes* plasmids were associated with cadmium resistance (Lebrun et al., 1992) or antibiotic resistance (Charpentier and Courvalin, 1999). Studies on the conjugative abilities of the plasmids associated with antibiotic resistance showed that they were transferable to *Listeria* spp. as well as to other Gram-positive bacteria and suggested that they were probably acquired from the *Enterococci-Streptococci* group (Poyart-Salmeron et al., 1990; Lebrun et al.,

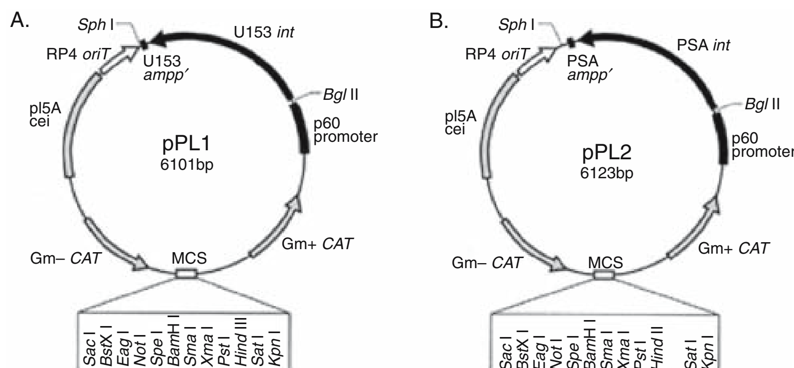

Fig. 7. Maps of the site-specific integration vectors pPL1 and pPL2. Chloramphenicol resistance genes (CAT) and origin of replication in *E. coli* are represented in gray; RP4 origin of transfer is represented in white. The U153 and PSA integrases (U153*int* and PSA*int*) and the p60 promoter of *L. monocytogenes* are represented in black. MCS is the multicloning site. Adapted from Lauer et al. (2002), with permission.

1992; Charpentier and Courvalin, 1999). Plasmid vectors originating from *Bacillus subtilis* or *Escherichia coli* replicate in *Listeria* and thus are used for genetic studies in *L. monocytogenes*, including allelic exchange of chromosomal DNA, cloning, gene expression, or reporter gene fusion (Freitag, 2000).

Transposons Some *L. monocytogenes* strains harbor a natural transposon Tn*5422*, which contains 40-bp inverted repeats, two genes conferring cadmium resistance, and two genes encoding a transposase and a resolvase (Lebrun et al., 1994). *Listeria* can also naturally acquire *Enterococci-Streptococci* transposons from the Tn*1545*-Tn*916* family, conferring on strains tetracycline resistance and other resistance genes (Poyart-Salmeron et al., 1989; Poyart-Salmeron et al., 1992). The conjugative properties of these transposons were widely used for mutagenesis and constituted the first genetic tools to study *L. monocytogenes* virulence and physiology (Gaillard et al., 1986; Kathariou et al., 1987; Portnoy et al., 1988; Cossart et al., 1989b; Marquis et al., 1993). Contrary to conjugative transposons, nonconjugative transposons such as Tn*917* or Tn*917-lac* do not generate multiple insertions and are consequently more powerful tools (Cossart et al., 1989b). More recently, Tn*1545* or Tn*917*–derived tagged transposons were also used for signature-tagged mutagenesis in *L. monocytogenes*, allowing the identification of new virulence genes (Autret et al., 2001; Dramsi et al., 2004; Mandin et al., 2004).

Genetic Techniques and Tools

Genetic techniques used to modify the *Listeria* genome are derived from those (including conjugation of self-conjugative transposons or plasmids, and transformation of plasmids) utilized to modify other Gram-positive bacteria (Cossart and Mengaud, 1989a). Interestingly, *L. monocytogenes* itself has been used as a genetic vehicle to deliver functional genes into eukaryotic cells (Mollenkopf et al., 2001; Grillot-Courvalin et al., 2002; Pilgrim et al., 2003b).

Plasmid Vectors Several shuttle plasmid vectors from *Bacillus subtilis* have been used in *L. monocytogenes*. They contain multicloning sites and genes encoding antibiotic resistance, allowing genetic studies (Freitag, 2000).

Plasmids Preferentially Used for Complementation or Gene Expression Among the broad host range shuttle plasmids replicating in *Escherichia coli* and *L. monocytogenes*, pMK4 and pAM401 are the most extensively used for complementation of mutants obtained by transposon insertion or for expression of gene products via their own promoters (Sullivan et al., 1984; Wirth et al., 1986; Cossart et al., 1989b; Freitag, 2000) (Fig. 8). Similarly, derivatives of the conjugative plasmid, pAT18 (Trieu-Cuot et al., 1991), allowed complementation of deletion mutants (Gaillard et al., 1991; Dramsi et al., 1995) as well as green fluorescent protein (GFP) expression (Fortineau et al., 2000). pBR474 derivatives have also been used to successfully express *L. monocytogenes* proteins. They combine the advantage of an easy selection of recombinants (because of the high natural sensitivity of *Listeria* strains to chloramphenicol) and a high level expression of inserts (because of the presence of a strong promoter) (Lecuit et al., 1997). The recent construction of a plasmid, pLIV1, carrying an isopropyl-β-D-thiogalactoside (IPTG)-inducible SPAC pro-

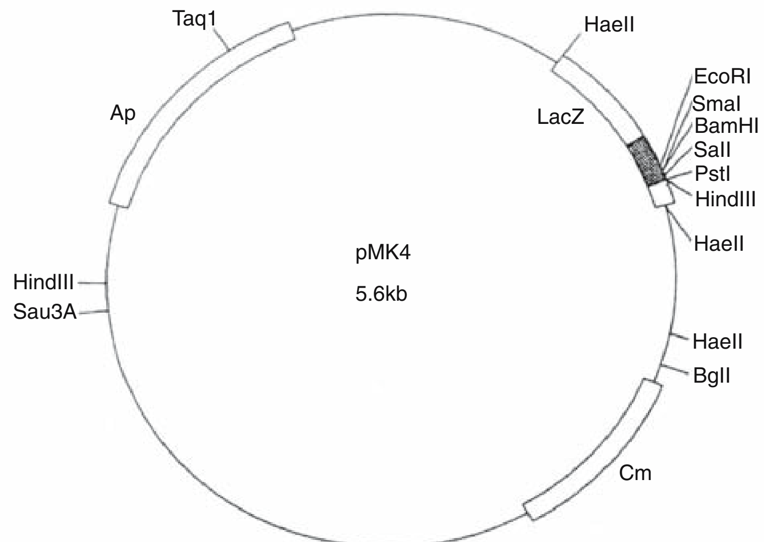

Fig. 8. Physical map of the pMK4 plasmid. The ampicillin (Ap) and chloramphenicol (Cm) resistance genes allow selection in *E. coli* and *L. monocytogenes*, respectively. From Sullivan et al. (1984), with permission.

moter permitted in vivo studies in which expression of *L. monocytogenes* genes were followed intracellularly (Freitag and Jacobs, 1999; Dancz et al., 2002). The construction of integrative phage-derived plasmids, pPL1 and pPL2, has also allowed complementation of deletion mutants (Fig. 7). Their advantage resides in their ability to integrate at a specific location, which may facilitate fine genetic analysis of mutants or gene transfer in *L. monocytogenes* (Lauer et al., 2002).

Plasmids Preferentially Used for Allelic Exchange Thermosensitive vectors such as pKSV7 (Smith and Youngman, 1992) (Fig. 9) or pAUL-A (Schaferkordt and Chakraborty, 1995) allow allelic exchange, in frame deletion mutagenesis (Brundage et al., 1993; Dramsi et al., 1995; Domann et al., 1997), or site-specific mutagenesis (Boujemaa-Paterski et al., 2001). A novel thermosensitive plasmid, pMAD, harboring the β-galactosidase gene fused to the strong constitutive promoter of *clpB* from *Staphylococcus aureus* (Fig. 10), facilitates the screening and the generation of allelic exchanges (Arnaud et al., 2004).

Transposons Different transposons were used for random mutagenesis in *L. monocytogenes*. Conjugative transposons from the Tn*1545*-Tn*916* family or Tn*917* transposons were initially exploited to generate libraries of mutants, which allowed the characterization of several virulence factors of *L. monocytogenes* (Gaillard et al., 1986; Kathariou et al., 1987; Portnoy et al., 1988; Cossart et al., 1989b). More recently, tagged Tn*1545* derivatives carried by an integrative vector were utilized for signature-tagged mutagenesis in *L. monocytogenes*, allowing the identification of new virulence genes (Autret et al., 2001). The use of Tn*917* or Tn*917* derivatives (pTV1, pTV32, pTLV1 and pTLV3), carried on vectors with temperature-sensitive origins, facilitated the screening and the generation of large-scale libraries of mutants, in particular those of *L. monocytogenes* virulence genes (Camilli et al.,

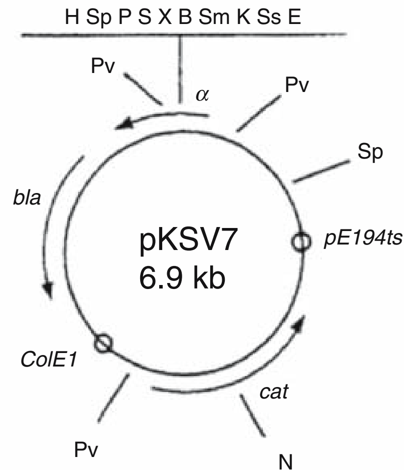

Fig. 9. Map of the pKSV7 thermosensitive plasmid. *bla* is the β-lactamase gene of pUC18; *cat* is the chloramphenicol acetylase transferase gene of pC194; α is the *lac* alpha-complementing gene fragment of pUC18. From Smith and Youngman (1992), with permission.

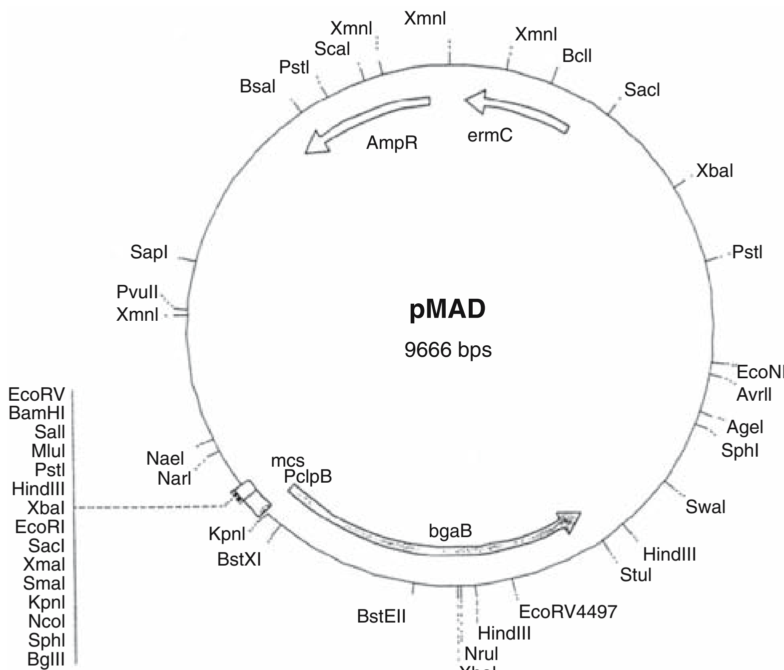

Fig. 10. Map of the pMAD plasmid. The ampicillin (*ampR*) and erythromycin (*ermC*) resistance genes allow selection in *E. coli* and *L. monocytogenes*, respectively. *bgaB* is the β-galactosidase gene fused to the strong constitutive promoter of *clpB* from *Staphylococcus aureus* (PclpB). From Arnaud et al. (2004), with permission.

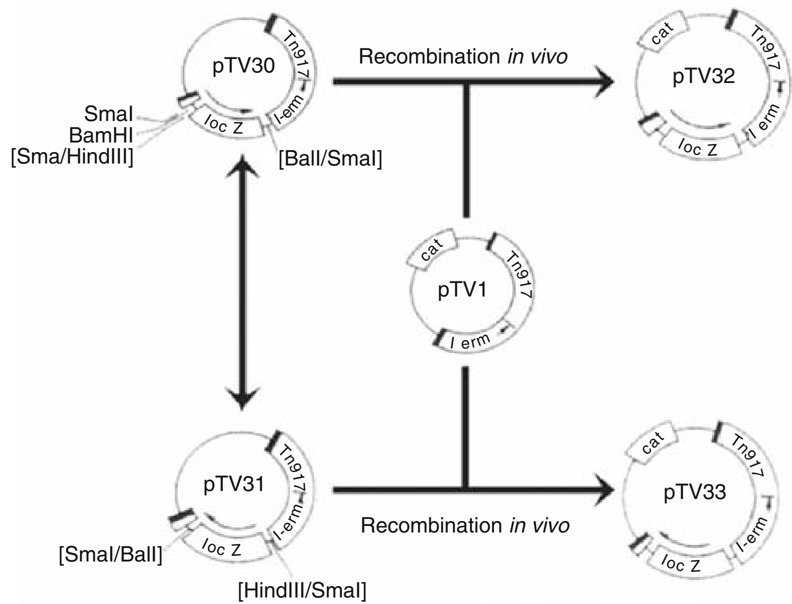

Fig. 11. Map of the pTV1, pTV31 and pTV32 plasmids. *erm* gene is the erythromycin resistance gene of the transposon Tn*917*; *cat* is the chloramphenicol acetylase transferase gene of pC194; *lacZ* is the promoterless *lacZ* gene of *E. coli*. From Perkins and Youngman (1986), with permission.

1990; Sun et al., 1990; Mengaud et al., 1991b) (Fig. 11). Some of these transposons have also the advantage of allowing the direct cloning of the flanking regions of the transposon insertion site (pTLV1 and pTLV3) or carry a promoterless *lacZ* gene permitting transcriptional gene fusion (pTV32) (Camilli et al., 1990; Francis and Thomas, 1997).

Reporter Genes Transcriptional fusions to reporter genes, mostly *lacZ*, *lux* or GFP, have been used to monitor *Listeria* gene expression in vitro or intracellularly (Klarsfeld et al., 1994; Bubert et al., 1999; Freitag, 2000). More recently, a transposon carrying a modified version of the *lux* operon fused to promoterless *L. monocytogenes* genes (Francis et al., 2000) has opened a new way to monitor infection using in vivo bioluminescence imaging in mice (Hardy et al., 2002).

Conjugation Self-conjugative transposons, plasmids, and phage-derived vectors are used for genetic studies in *Listeria*. Most often, conjugation is performed on filters onto which donor and recipient bacteria are deposited (Cossart and Mengaud, 1989a). pAT18 vectors or derivatives were used for complementation studies of mutants affected in virulence determinants (Gaillard et al., 1991) or to generate *L. monocytogenes* expressing GFP, allowing their detection and tracking in vivo (Fortineau et al., 2000). Conjugative transposons of the Tn*1545*-Tn*916* family transfer from the donor bacteria to the recipient bacteria, where they integrate randomly into the chromosome. This strategy was the first used to generate a large set of defective mutants (Gaillard et al., 1986; Kathariou et al., 1987; Portnoy et al., 1988; Cossart et al., 1989b; Marquis et al., 1993). Recently, conjugation of listeriophage-derived integrative vectors, pPL1 and pPL2, was successfully exploited for complementation studies of *L. monocytogenes* mutants (Lauer et al., 2002).

Transformation Listeriae are not naturally competent, although competence genes have been found in *L. monocytogenes* genome (Glaser et al., 2001). However, transformation with plasmid DNA can be obtained on protoplasts or by electroporation (Vicente et al., 1987; Luchansky et al., 1988). Treatment of bacteria with low concentration of penicillin optimizes the efficiency of electroporation (Park and Stewart, 1990). Genetic studies resulting from *L. monocytogenes* transformation include allelic exchange of chromosomal DNA, complementation of defective genes, and reporter gene fusion (Cossart et al., 1989b; Dramsi et al., 1995; Dubail et al., 2000).

Transduction Until recently, transduction was not possible in *L. monocytogenes*. It has now been successfully performed using bacteriophages that grow at 30°C but not at 37°C. Transduction at low multiplicity of infection, at 37°C, and in presence of citrate resulted in elimination of transductant and lysogeny, allowing the isolation of several *L. monocytogenes* transductants of different serotypes, and opening the way to new genetic approaches to modify the *Listeria* genome (Hodgson et al., 2000).

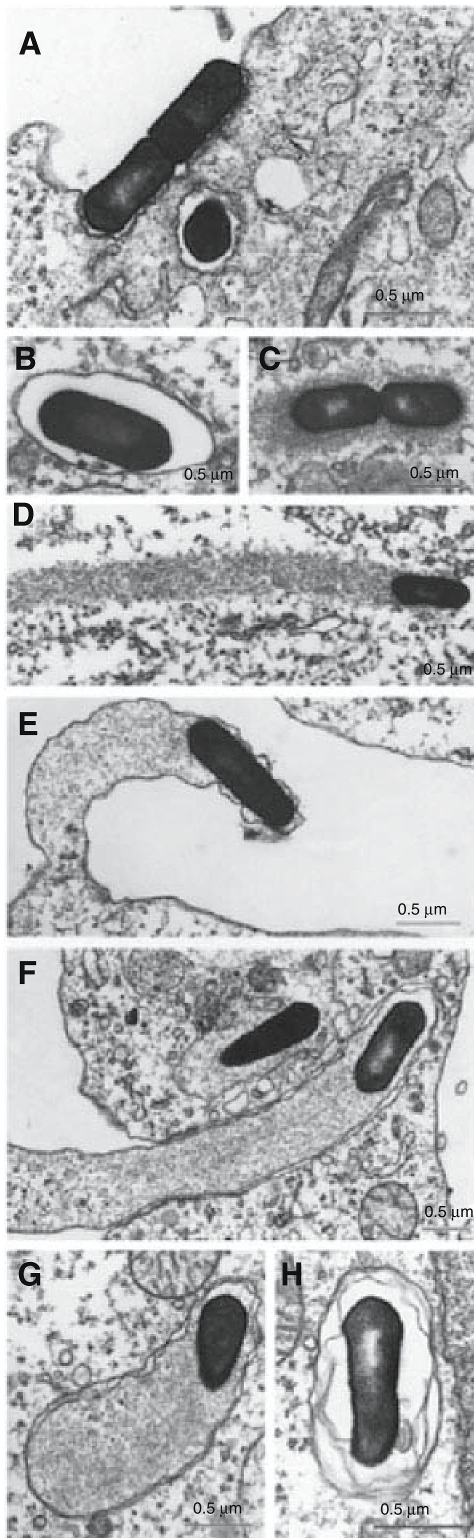

Fig. 12. Electron microscopy images of the successive steps of *L. monocytogenes* infection. Thin sections of cells infected with *L. monocytogenes* are examined by electron microscopy. A and B: entry and formation of the phagocytic cup; C and D: intracellular movements; E and F: cell-to-cell spread; G and H: formation and lysis of the two-membrane vacuole. From Cossart and Lecuit (1998), with permission.

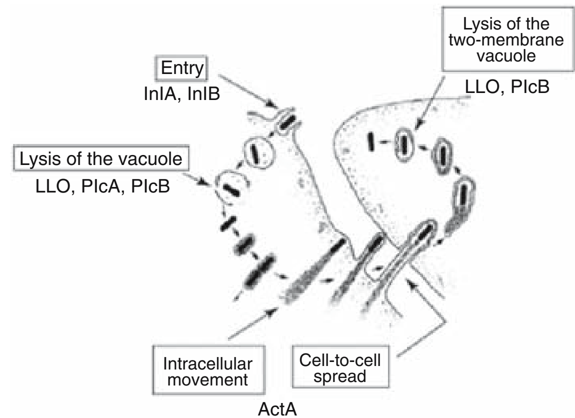

Fig. 13. Model for *L. monocytogenes* infection. The successive steps of *L. monocytogenes* infection as well as the bacterial factors are indicated. Adapted from Tilney and Portnoy (1989), with permission.

The Cell Biology of *L. monocytogenes* Infection

While our knowledge of the different steps leading to listeriosis remains rather elusive, the cell biology of the infectious process has been widely studied (Figs. 12 and 13). The main feature of *L. monocytogenes* is its capacity to enter into nonphagocytic cells and to survive and multiply in the cytosol of most cell types, including macrophages. Intracytoplasmic bacteria exploit the host cytoskeleton to move intracellularly through an actin-dependent process. (Lecuit and Cossart, 2001; Cossart and Sansonetti, 2004). Interestingly, it was shown that intracytosolic *L. monocytogenes* avoids recognition by the ubiquitin-dependent degradation system, as a possible consequence of its motility (Perrin et al., 2004). *L. monocytogenes* form protrusions that invade adjacent cells, allowing dissemination by direct cell-to-cell spread, without reaching the extracellular milieu, therefore avoiding the extracellular defenses, such as antibodies and complement (Lecuit and Cossart, 2001a; Cossart and Sansonetti, 2004). These features, including the avoidance of lysosomal enzymes and serum components, strongly account for the virulence of *L. monocytogenes*.

Bacterial Internalization

Both in vivo and in cultured cells, *L. monocytogenes* can be phagocytosed by macrophages (Harrington-Fowler et al., 1981; Campbell et al., 1994), neutrophils (Arnold and Konig, 1998), or dendritic cells (Guzman et al., 1995; Kolb-Maurer et al., 2000; Pron et al., 2001). *Listeria monocytogenes* can also induce its own internalization in a wide variety of nonphagocytic cells,

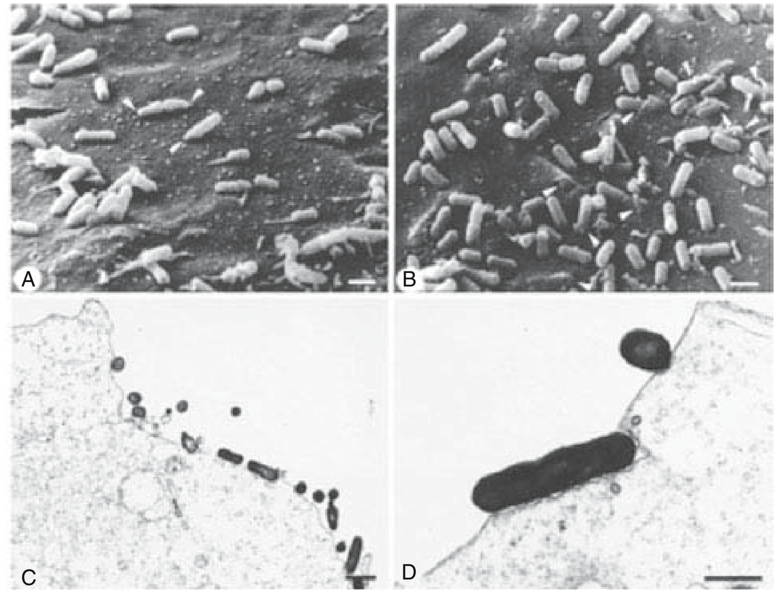

Fig. 14. Electron microscopy of *L. monocytogenes* invading Caco-2 cells. Scanning electron microscopy images of cells infected for 15 min (A) or 30 min (B) by *L. monocytogenes*. Transmission electron microscopy images of cells infected by *L. monocytogenes* at low (C) or high (D) magnification. Scale bar, 0.5 μm. From Mengaud et al. (1996), with permission.

including enterocytes (Gaillard et al., 1987; Lecuit et al., 2001b), hepatocytes (Gaillard et al., 1996; Gregory et al., 1996b), fibroblasts (Portnoy et al., 1988), endothelial cells (Drevets et al., 1995; Greiffenberg et al., 1998; Parida et al., 1998) or glial cells of the CNS (Dramsi et al., 1998). Phagocytosis of *L. monocytogenes* in epithelial cells does not produce an extensive remodeling of the cell surface, as seen with *Salmonella*- or *Shigella*-induced ruffling (Swanson and Baer, 1996). Rather it occurs by a zipper-like mechanism, characterized by an intimate bacterium-cell membrane interaction, followed by the progressive invagination of the plasma membrane leading to the bacterial engulfment into the adjacent cell (Mengaud et al., 1996; Figs. 12A and 14). *Listeria* entry into non-phagocytic cells was shown to be dependent on membrane cholesterol (Seveau et al., 2004) and to exploit the clathrin-dependent endocytic cell machinery (Veiga and Cossart, 2005). The entry process is predominantly promoted by two leucine-rich repeat (LRR) proteins, InlA and InlB, expressed at the surface of *L. monocytogenes* and belonging to the internalin (Inl) multigenic family (Gaillard et al., 1991; Dramsi et al., 1995; Braun et al., 1999).

Intracellular Multiplication

After phagocytosis, the vacuole containing *L. monocytogenes* acidifies and, most often, is lysed by the bacterium in less than 30 min (Gaillard et al., 1987; Tilney and Portnoy, 1989; Fig. 12B). In macrophages, escape from the phagosome occurs for approximately 50% of the bacterial population and prevents bacterial destruction by the phagolysosomal components (Tilney and Portnoy, 1989; De Chastellier et al., 1994). It involves principally three bacterial proteins, the pore forming toxin, LLO (Gaillard et al., 1987), PLC-A (Camilli et al., 1993), and the phosphatidylcholine (PC) phospholipase C (PLC-B) (Grundling et al., 2003). Recently, it was suggested that lipoproteins might also participate in the bacterial escape from the phagosome (Reglier-Poupet et al., 2003a). In macrophages, *L. monocytogenes* delays phagosome maturation, which probably accounts for the vacuolar escape (Alvarez-Dominguez, 1997a). *L. monocytogenes* also inhibits the exchange activity of the endosomal trafficking regulator Rab5a, blocking the recruitment of lysosomal proteins to the phagosomes and avoiding the intraphagosomal killing of *L. monocytogenes* (Prada-Delgado et al., 2001; Prada-Delgado et al., 2005). However, acidification of the phagosomes containing *L. monocytogenes* occurs and is required for membrane disruption induced by the bacteria (Beauregard et al., 1997). In non-phagocytic cells, phagosomes containing InlA- or InlB-coated latex beads contain not only endosomal-lysosomal markers but also MSF, which is a member of the septin family involved in membrane fusion events (Pizarro-Cerda et al., 2002).

Escape from phagosomes involves principally three bacterial proteins, the pore forming toxin, LLO (Gaillard et al., 1987), the phosphatidylinositol (PI) phospholipase (PLC-A) (Camilli et al., 1993), and the phosphatidylcholine (PC) phospholipase C (PLC-B) (Grundling et al., 2003). It was suggested that lipoproteins might also participate in the bacterial escape from the phagosome (Reglier-Poupet et al., 2003a).

Once free in the cytosol, *L. monocytogenes* starts multiplying, with an approximate doubling

time of one-hour (Tilney and Portnoy, 1989; Fig. 12C). This is true for most cells studied, but certain listericidal macrophages do not permit intracellular growth, especially when phagocytosis occurs through the C3bi complement receptor CR3 (Drevets et al., 1993). Several genes encoding virulence or metabolic determinants are induced during *L. monocytogenes* intracellular life, including those involved in phagosomal lysis, actin-based motility, and cell-to-cell spreading (Klarsfeld et al., 1994; Freitag et al., 1999; Bubert et al., 2000). Unlike most bacteria, *L. monocytogenes* replicates in the cytosol when it is directly microinjected into cells (Goetz et al., 2001). The cytosol permissiveness for *L. monocytogenes* growth is probably due to its ability to use a variety of cytosolic nutrients, as suggested by the fact that intracellular multiplication of several auxotrophic mutants is not affected (Marquis et al., 1993). Intracytosolic growth of *L. monocytogenes* is dependent on the hexose phosphate transporter *hpt* gene. Expression of *htp* is under the control of the transcriptional activator PrfA (positive regulatory factor A), which regulates most virulence genes (Goetz et al., 2001).

Actin-Based Motility

Upon escape from the phagosome, *L. monocytogenes* gets surrounded by actin filaments and actin binding proteins. The bacteria start moving in the cytosol, where they induce the formation of an actin tail at their rear end, which resembles a comet constantly depolymerizing at its distal end (Tilney and Portnoy, 1989; Cossart and Kocks, 1994; Figs. 12D, 15 and 16). *Listeria monocytogenes* comets are composed of a dense meshwork of cross-linked actin filaments, similar to that induced by *Shigella flexneri* but different from that induced by *Rickettsia conorii*, which are composed of unbranched actin filaments (Gouin et al., 1999, 2005) (Fig. 16). Actin tails are on average 5 µm but can be as long as 40 µm (Tilney and Portnoy, 1989). *Listeria monocytogenes* motility results from the polymerization of cellular actin by the ActA protein expressed at one pole of the bacterial body (Tilney and Portnoy, 1989; Domann et al., 1992; Kocks et al., 1992). The speed of moving bacteria correlates with the length of the comet and is 0.25 µm/s on average. The process of actin polymerization is very dynamic, since the actin tail is constantly polymerizing and depolymerizing, generating the forces to push the bacteria forward (Theriot et al., 1992). Occasionally, propelled bacteria encounter a membrane and form a protrusion (Fig. 12E). When it occurs in the vicinity of an adjacent cell, it allows bacterial dissemination from cell to cell without

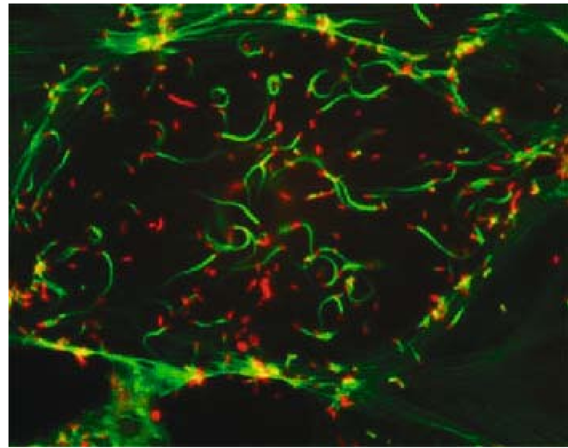

Fig. 15. Actin comets formed in PTK2 after infection with *L. monocytogenes*. PTK2 cells are infected with *L. monocytogenes* for 5 h and fixed. Actin is stained with fluorescein-phalloidin (green) and *L. monocytogenes* are labeled with an anti-*L. monocytogenes* antibody detected by a secondary rhodamine-labeled antibody (red). From N. Khelef, unpublished image.

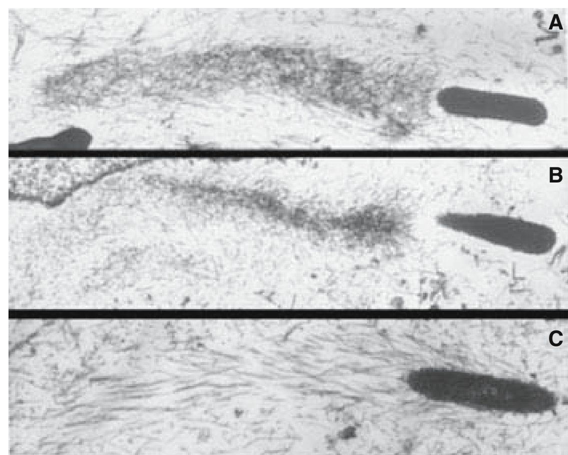

Fig. 16. Electron micrograph of myosin S1 decorated actin comets formed by *L. monocytogenes* (A), *Shigella flexneri* (B) and *Rickettsia conorii* (C) in Hep-2 cells. Adapted from Gouin et al. (1999), with permission.

reaching the extracellular milieu, therefore avoiding the humoral immune response (Tilney and Portnoy, 1989) (Fig. 12F). The actin tail is composed of actin and actin binding proteins. These include the Arp2/3 complex, which is involved in actin nucleation and polymerization (Welch et al., 1997), and VASP, the vasodilator-stimulated phosphoprotein, which directly binds actin (Chakraborty et al., 1995). The actin tail also colocalizes with proteins involved in actin depolymerization, such as cofilin (David et al., 1998; Gouin et al., 1999), or the actin-bundling protein α-actinin (Dabiri et al., 1990) (Fig. 17). The process of *L. monocytogenes* actin based motility involves an Arp2/3-dependent nucleation step and an Arp2/3-independent step,

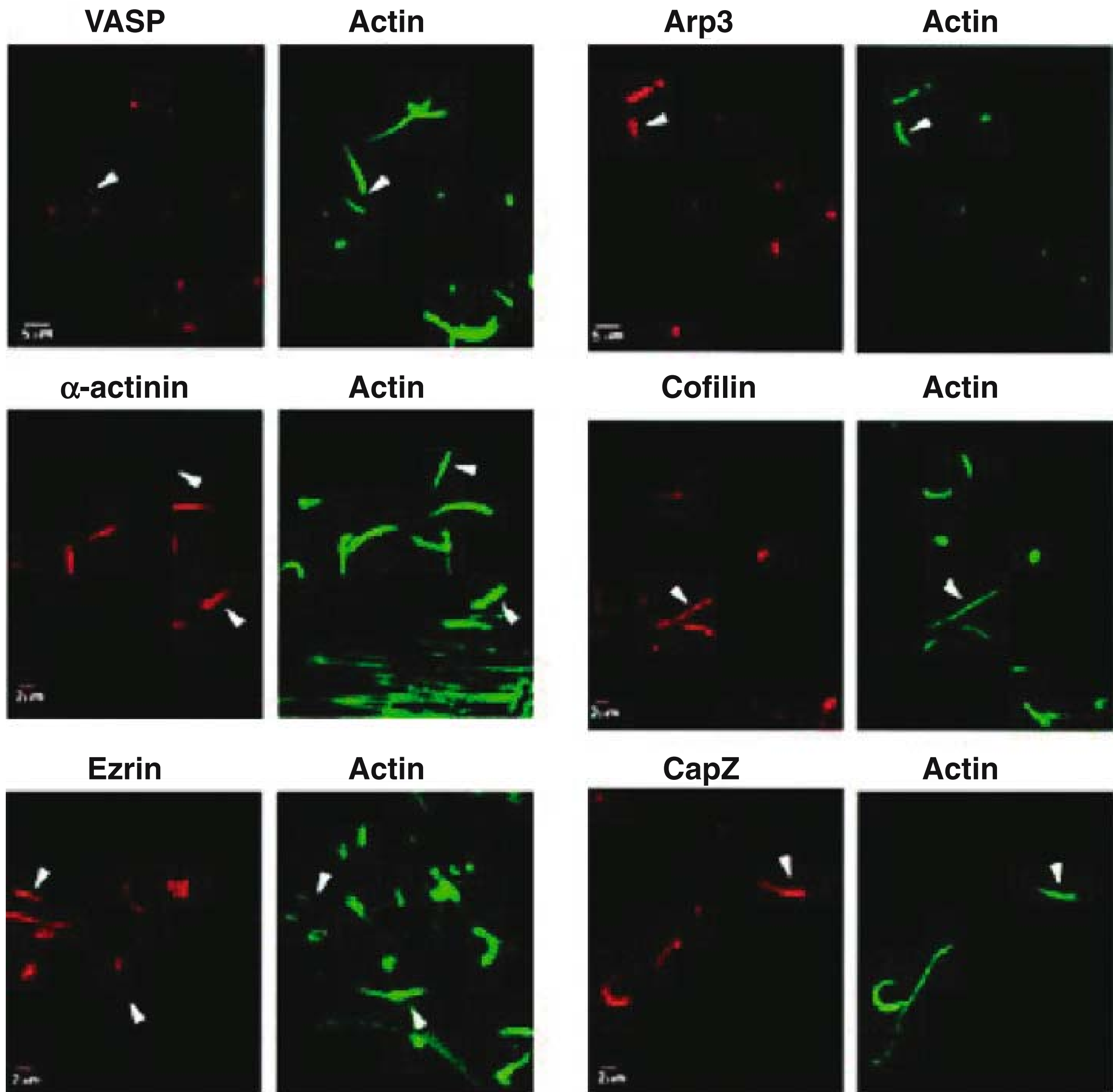

Fig. 17. Localization of the actin partners during the formation of comets in cells infected by *L. monocytogenes*. Actin is labeled with fluorescein isothiocyanate-phalloidin or bodipy-phallacidin (green). Vasodilator-stimulated phosphoprotein (VASP), α-actinin, ezrin, actin-related protein Arp3, cofilin and capZ (capping protein Z) are labeled using specific antibodies detected with secondary fluorescent antibodies (red). Images are acquired by confocal scanning microscopy. From Gouin et al. (1999), with permission.

which requires fascin, an actin bundling protein (Brieher et al., 2004). In addition, it was shown that PI-3K plays a role in *L. monocytogenes* action motility (Sidhu et al., 2005). Using green fluorescent protein fusions proteins (pleckstrin homology domains of phospholipase C and of Akt, respectively), which bind PI(4,5)P2 and PI(3,4,5)P3 or PI(3,4)P2, to detect these phosphoinositides, it was shown that phosphoinositides are recruited around the moving bacteria and then concentrate at the comet tail, explaining the involvement of PI3-K in *L. monocytogenes* motility (Sidhu et al., 2005). The actin-based motility of *L. monocytogenes* is a good example of the exploitation of the host cell machinery by pathogenic bacteria to promote their own survival (Cossart, 1997). Moreover, this model was efficiently exploited for a better comprehension of complex cellular processes such as the formation of the leading edge during cell migration (Machesky, 1997; Marx, 2003).

Intercellular Spreading

During intracellular multiplication within primary infected cells, *L. monocytogenes* generates an actin comet tail, which propels the bacteria in random directions and occasionally to the cell

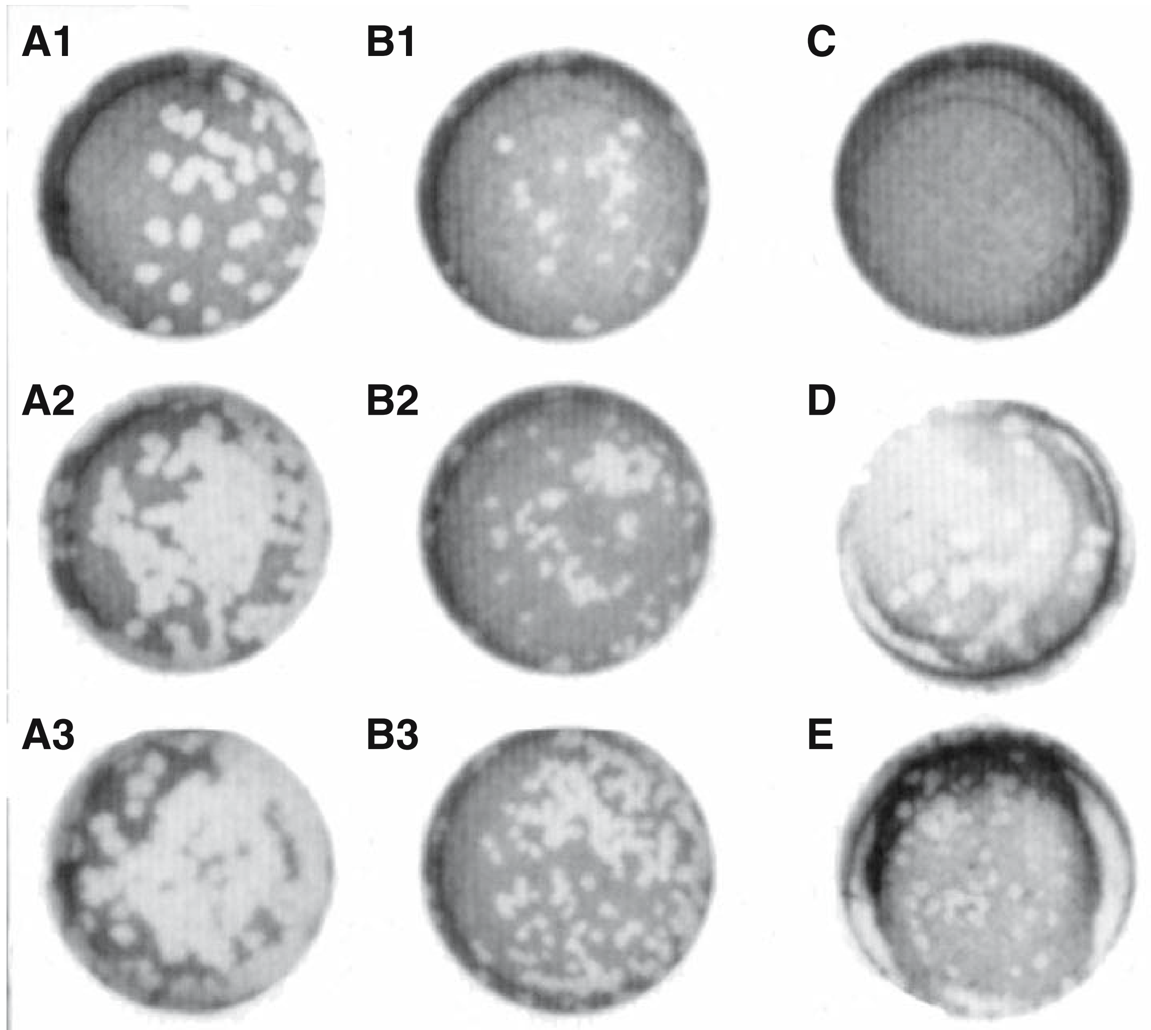

Fig. 18. Plaque formation in 3T3 fibroblasts produced by *L. monocytogenes* infection. Cells were infected with increasing amounts (A1 to A3 and B1 to B3) of *L. monocytogenes* wild-type or *plcB* mutant, or with similar amounts of *L. monocytogenes* wild-type (D), *actA* mutant (C) or *actA* complemented mutant (E). Plaques were visualized by Giemsa staining after 3 days infection. Adapted from Kocks et al. (1992), with permission.

periphery, generating a protrusion, which contains the bacteria. If the moving bacterium encounters the membrane of an adjacent cell, the protrusion invaginates in this cell, generating a double membrane vacuole containing *L. monocytogenes* and part of the actin tail (Fig. 12F and G). The vacuole of the secondarily infected cell is then lysed liberating bacteria into the cytosol and starting a new infectious cycle (Tilney and Portnoy, 1989). Lysis of the double membrane requires both listeriolysin O (LLO) and the phosphatidylcholine phospholipase C (PLC-B), which is matured by the metalloprotease, Mpl (Mengaud et al., 1991c; Domann et al., 1999; Vazquez-Boland et al., 1992; Smith et al., 1995a). The direct cell-to-cell spreading of *L. monocytogenes* can be followed in a plaque formation assay. In this assay, a fibroblast monolayer is infected, covered by an agarose layer containing gentamicin, which kills extracellular bacteria. After a few hours, plaques corresponding to islets of cells killed by the bacteria can be visualized by Giemsa or crystal violet staining (Kocks et al., 1992; Brouqui et al., 1994; Fig. 18). Their size is proportional to the bacterial ability to infect adjacent cells and disseminate in the monolayer (Havell, 1986). At the molecular level, it was shown that ezrin, a membrane-cytoskeleton linker of the ERM (ezrin-radixin-moesin) family, accumulates at *Listeria* protrusions. Interaction of ERM proteins with membrane components and actin is required for the formation of protrusions and for efficient cell to cell spread (Pust et al., 2005). The *L. monocytogenes* protein ActA plays a key role in cell-to-cell spread since it is required for the efficient actin polymerization responsible for the bacterial motility (Kocks et al., 1992; Cossart and Bierne,

2001). Cell-to-cell spread is an important feature of *L. monocytogenes* pathogenicity since mutants unable to spread from cell to cell is strongly attenuated (Barry et al., 1992). This ability to spread from cell to cell allows bacterial dissemination through an epithelial tissue without reaching the extracellular medium and its antibacterial products including antibodies.

Virulence Factors

Several proteins produced by *L. monocytogenes* are involved in virulence, either in animal or in cellular models. Most of them were identified by transposon mutagenesis in the 1990s (Gaillard et al., 1986; Gaillard et al., 1991; Kathariou et al., 1987; Cossart et al., 1989b; Mengaud et al., 1991b; Kocks et al., 1992). Recently, new techniques, including signature tagged mutagenesis and comparative genomics between pathogenic *L. monocytogenes* and nonpathogenic *L. innocua* isolates, led to the identification of new virulence factors or putative ones (Autret et al., 2001; Glaser et al., 2001; Cabanes et al., 2002, 2004; Dussurget et al., 2002, 2004a; Dramsi et al., 2004; Mandin et al., 2005).

The best characterized virulence factors of *L. monocytogenes* are involved in the four steps of the cell infectious cycle. Entry into epithelial cells is mediated by the internalins InlA and InlB (Gaillard et al., 1991; Dramsi et al., 1995). Lysis of the primary vacuole involves LLO, PLC-A and PLC-B (Gaillard et al., 1987; Camilli et al., 1993; Grundling et al., 2003). Intracellular motility is promoted by the ActA protein (Domann et al., 1992; Kocks et al., 1992). Intercellular spreading involves the ActA protein, LLO, PLC-B, and Mpl, which activates PLC-B (Mengaud et al., 1991c; Kocks et al., 1992; Vazquez-Boland et al., 1992; Smith et al., 1996). Expression of these virulence factors is controlled by the transcriptional regulator PrfA (Kreft and Vazquez-Boland, 2001).

Internalins

Internalins belong to a multigenic family of proteins characterized by a N-terminal domain containing several successive LRR of 22 amino acids. They harbor a signal peptide and are therefore exported at the cell surface (Gaillard et al., 1991; Dramsi et al., 1997; Engelbrecht et al., 1998a; Raffelsbauer et al., 1998). Nine internalins were first identified in the strain EGD (InlA to InlH and InlC2) (Gaillard et al., 1991; Dramsi et al., 1997; Engelbrecht et al., 1998a; Raffelsbauer et al., 1998). The determination of the genome sequence of the strain EGDe then revealed the presence of larger numbers of internalin or internalin-like genes in the strain EGDe (Cabanes et al., 2002) (Fig. 19). The internalin family can be divided into three classes. The first class comprises internalins containing a LPXTG motif in their C-terminal region (LPXTG proteins), through which they are covalently anchored to the cell wall (Fig. 20). InlA is the best characterized member of this protein family, which contains 18 other members in strain EGDe (Fig. 19A). The second class comprises one internalin in strain EGDe, InlB, which contains a C-terminal region of 80 amino-acid repeats starting with the dipeptide GW modules (Fig. 19B). GW modules mediate a loose association of InlB to the bacterial surface (Fig. 20). A third class is composed of internalins that do not display any surface targeting domain and are therefore secreted in the extracellular medium. InlC is the best characterized member of this family (Fig. 19C). The two first groups of internalins also contain a region of repeated sequences, named B repeats (Vazquez-Boland et al., 2001; Cabanes et al., 2002).

The first internalins to be discovered, InlA and InlB, are the major effectors of *L. monocytogenes* entry into nonphagocytic cells (Gaillard et al., 1991; Dramsi et al., 1995). The functions of the other internalins remain unclear. Single or multiple deletions of internalin genes, including *inlC*, *inlC2-inlD-inlE* (in strain EGD) or *inlG-inlH-inlE* (in strain EGDe), decreased the virulence of *L. monocytogenes* but did not affect entry into epithelial cells or intracellular multiplication (Domann et al., 1996; Dramsi et al., 1997; Raffelsbauer, 1998). This suggests a role for one of these genes in pathogenicity besides invasion. Deletion of the *inlF* gene has no effect on bacterial entry and virulence (Dramsi et al., 1997). A recent study examining double mutants affecting different internalin genes, including, *inlA*, *inlB*, *inlG*, *inlH* and *inlE*, showed that efficient InlA-dependent entry requires the functions of InlC and InlG-InlH-InlE, suggesting a cooperation between the various internalins for efficient cell invasion (Bergmann et al., 2002), as previously shown for InlA and InlB. The presence of multiple internalins suggests that they may also be important for the biology of *L. monocytogenes* besides pathogenesis.

Genetic Organization and Transcription of Internalin Genes Internalin genes are dispersed along the chromosome, but some of them are associated in clusters, such as the operon *inlAB* or the clusters *inlG-inlC2-inlD-inlE* or *inlG-inlH-inlE* in *L. monocytogenes* (Vazquez-Boland et al., 2001). Gene rearrangements are suggested by the fact that one internalin locus is different in two other *L. monocytogenes* EGD isolates. Indeed, comparison of the *inlG-inlC2-*

Fig. 19. Alignment of internalin-like LPXTG proteins (A), GW proteins (B), and InlC-like proteins (C). Determined from the annotation of the *L. monocytogenes* genome sequence. The numbers within domains represent the number of repeats. From Cabanes et al. (2002), with permission.

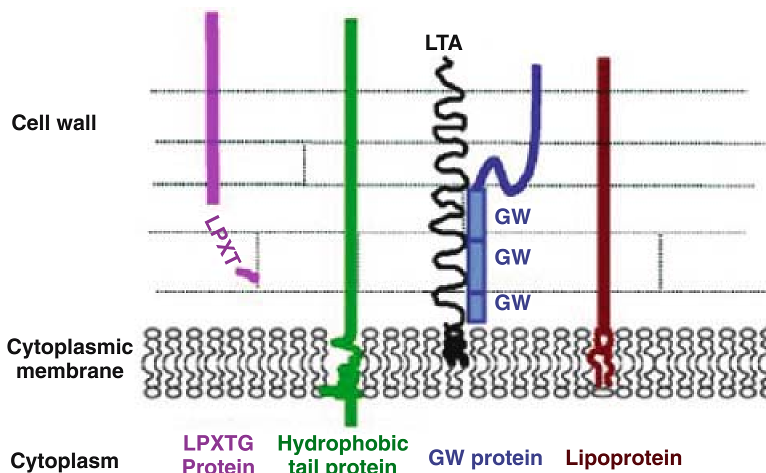

Fig. 20. Association of *L. monocytogenes* surface proteins. Some surface proteins are covalently anchored to the cell wall through a LPXTG motif or loosely attached to the LTAs (lipoteichoic acids) through GW domains. Others are anchored to the cytoplasmic membrane through a hydrophobic tail or a lipid anchor (lipoproteins). From Cabanes et al. (2002), with permission.

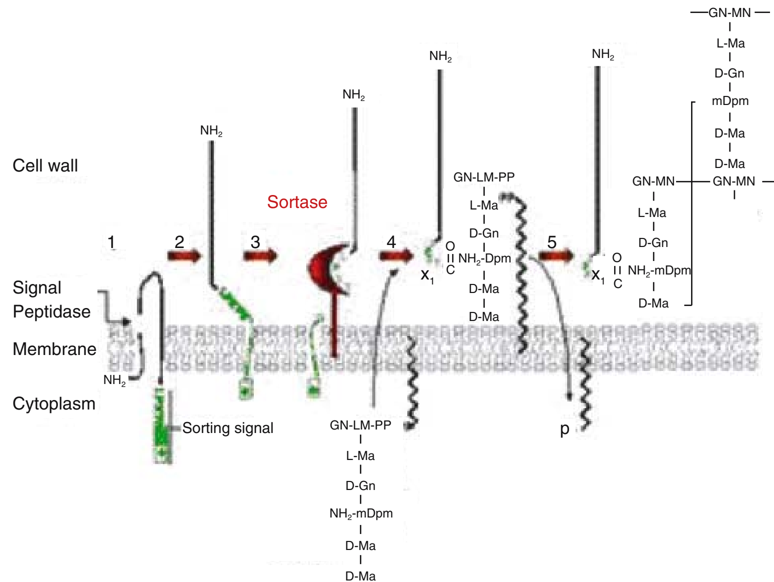

Fig. 21. Model for cell wall sorting. The precursor is exported from the cytoplasm via an N-terminal signal sequence (1). The protein is transiently retained in the cytoplasmic membrane by its charged tail and hydrophobic domain (2) allowing recognition and processing of the LPXTG motif by sortase A between the threonine and the glycine residues (3). Sortase A catalyzes the formation of an amide bond between the carboxyl group of threonine and the amino group of the *m*-diaminopimelic acid (DAP) of the cell wall peptidic cross-bridges within a peptidoglycan precursor (4). The precursor is then incorporated into the cell wall by transpeptidation and transglycosylation reactions (5). Adapted from Navarre and Schneewind (1999), with permission.

inlD-inlE locus of strain EGD and *inlG-inlH-inlE* locus of another isolate of strain EGD showed that the *inlH* gene probably resulted from the rearrangement between *inlC2* and *inlD* (Dramsi et al., 1997; Raffelsbauer et al., 1998). The *inlA* and *inlB* genes can be transcribed individually or cotranscribed as an operon (Lingnau et al., 1995; Dramsi et al., 1997). Transcription of the *inlA* gene occurs from three promoters, but only one of them is controlled by the transcriptional activator PrfA, which regulates several virulence factors. This PrfA-dependent promoter also controls *inlB* gene expression (Lingnau et al., 1995; Sheehan et al., 1995; Dramsi et al., 1997). Several internalins (including InlA, InlB, InlC2, InlD and InlE) are also positively regulated by the sigma B factor (Kazmierczak et al., 2003). Transcription of the *inlC* gene is also strongly dependent on the PrfA regulator (Engelbrecht et al., 1996), while the *inlGHE* genes are transcribed individually from a major PrfA-independent promoter (Raffelsbauer et al., 1998).

Anchoring of Internalins The various surface proteins of *L. monocytogenes* are associated to the bacterial surface, by interacting with either the plasma membrane, peptidoglycan, or cell wall secondary polymers (Fig. 20). In Gram-positive bacteria, anchoring of LPXTG proteins is mediated by sortase A (SrtA), which catalyzes the covalent linkage of the LPXTG motif to the peptidoglycan, after cleavage of the T-G bond of this motif (Mazmanian et al., 1999; Mazmanian et al., 2001; Navarre and Schneewind, 1999) (Fig. 21). In *L. monocytogenes*, deletion of the SrtA gene abolishes the anchoring of most peptidoglycan associated proteins, including InlA (Bierne et al., 2002b; Garandeau et al., 2002). In the mouse model of infection, after injection by intravenous or oral routes, the virulence of a SrtA-deficient mutant is attenuated, as compared with the isogenic parental strain and with an InlA-deficient mutant. This suggests that LPXTG proteins other than InlA are required for full virulence in this animal model (Bierne et al., 2002b). InlB is not covalently anchored to the peptidoglycan by a LPXTG motif but instead possesses a 232-amino acid region made of two long tandem repeats starting with the dipeptide GW (Fig. 20). These GW modules are responsible for the loose association of InlB to the bacterial membrane through direct interaction with the lipoteichoic acids (LTAs). In addition, the GW modules of InlB interact with two host cell receptors, the glycosaminoglycans (GAGs) and the complement receptor gC1qR (Braun et al., 1997; Jonquieres et al., 1999; Braun et al., 2000; Marino et al., 2002). InlC and InlC-like proteins lack the LPXTG motif, as well as the B repeat region, and are therefore secreted by *L. monocytogenes* (Engelbrecht et al., 1996).

LRRs Motifs of Internalins The LRRs motifs are often involved in protein-protein interactions. This was demonstrated in the interaction of both InlA and InlB with their specific cellular receptors, respectively the human E-cadherin (Lecuit et al., 1997) and the Met receptor (Shen et al., 2000). In prokaryotes, LRR motifs are present in different virulence factors (Table 5). In eukaryotes, LRR proteins are involved in protein-protein interactions in various cellular processes, including recognition of bacterial pathogens by the Toll-like receptors and Nod proteins (Barton and Medzhitov, 2003; Chamaillard et al., 2003), as well as in plant pathogen defenses (Rathjen and Moffett, 2003).

Table 5. Virulence factors containing LRR motifs.

Names	Pathogens	Involved in	References
FHA	*Bordetella pertussis*	Adhesion	Makhov et al., 1994
YopM	Yersiniae	Cellular kinase activation	Leung and Straley, 1989 McDonald et al., 2003
IpaH	*Shigella flexneri*	Phagosomal escape	Hartman et al., 1900
Slr	Group A *Streptococcus*	Adhesion and phagocytosis	Reid et al., 2003
SlrP	*Salmonella typhimurium*	Host adaptation	Tsolis et al., 1999
SspH2	*Salmonella typhimurium*	Cytoskeleton binding protein	Miao et al., 2003
SspH1	*Salmonella typhimurium*	Inhibition of NF-κB pathway	Haraga and Miller, 2003

Abbreviations: LRR, leucine-rich repeats; FHA, filamentous hemagglutinin; YopM, *Yersinia* outer protein M; IpaH, invasion plasmid antigen; Slr, streptococcal leucine-rich; Ssp, salmonella serine protease.

This similarity could be used as a potential molecular mimicry of the host cell system by the microorganisms.

Cellular Specificity of InlA and InlB Both InlA and InlB promote invasion of nonphagocytic cells when coated onto latex beads or expressed by noninvasive bacteria, demonstrating that they are both sufficient for entry (Lecuit et al., 1997; Braun et al., 1998). However, they have different cell specificity, probably due to the presence of their respective receptors on different cell types. InlA mediates entry into cells expressing its receptor, the human E-cadherin, as first shown in vitro for the human enterocyte-like epithelial cell line, Caco-2, and for certain hepatocyte cell lines, such as HepG-2, or placental cells (Dramsi et al., 1995; Mengaud et al., 1996; Lecuit et al., 1997; Lecuit et al., 2004; Bakardjiev et al., 2004). Studies on InlA-dependent entry are now often performed using embryonic fibroblast cells L2071 transfected with the human E-cadherin (Lecuit et al., 2000). In contrast, InlB promotes invasion into several cell types, including certain epithelial cell lines (HeLa, Hep-2 and Vero), hepatocytes (HepG2 and TIB73), brain microvascular endothelial cells (HBMEC), endothelial cells (HUVEC) and fibroblasts (Dramsi et al., 1995; Lingnau et al., 1995; Gregory et al., 1997; Greiffenberg et al., 1998; Parida et al., 1998). Entry through InlA and InlB-dependent pathways were shown to be both dependent on plasma membrane cholesterol, although at different molecular steps (Seveau et al., 2004).

Interaction of InlA with its Receptor E-cadherin and Consequences InlA is the major factor required for *L. monocytogenes* entry into the human enterocyte-like epithelial cell line Caco-2 (Gaillard et al., 1991; Dramsi et al., 1995). When coated onto beads or expressed in non-invasive bacteria, InlA is sufficient to promote invasion of nonphagocytic cells expressing cadherin. Structure function studies showed that the LRRs and the inter-repeat regions of InlA are sufficient to promote entry into epithelial cells (Lecuit et al., 1997).

Interaction with E-cadherin The receptor for InlA was identified by affinity chromatography as the human E-cadherin, an intercellular adhesion protein, which is highly expressed at the basolateral membrane, in adherens junctions, and possibly on the apical membrane of polarized epithelial cells (Mengaud et al., 1996). E-cadherin is also expressed in hepatocytes, microvascular endothelial cells and choroid plexus cells in the CNS, cytotrophoblastic cells at the placental level, and dendritic cells, suggesting that InlA may target these cells during infection. Interaction of InlA with E-cadherin is the key step in bacterial entry into epithelial cells and in the formation of the phagocytic cup at the entry site. The extracellular domain of E-cadherin interacts with the LRR domain of InlA. *Listeria monocytogenes* recruits α-catenin at its entry site to create a link between E-cadherin and the cytoskeleton through β- and α-catenins (Lecuit et al., 1999; Lecuit et al., 2000) (Fig. 22). Vezatin mediates the bridge between the cadherin-catenins complex and the unconventional myosin VIIa (Kussel-Andermann et al., 2000). Vezatin and myosin VIIa are recruited to the *L. monocytogenes* entry site (Fig. 23) and are involved in the cytoskeleton rearrangements required for efficient InlA-dependent phagocytosis of beads or bacteria (Sousa et al., 2004) (Fig. 24).

Specificity of the InlA-E-cadherin Interaction The InlA-E-cadherin interaction is species specific, since InlA recognizes the human E-cadherin but not the mouse E-cadherin. This specificity is due to a single amino acid, a proline located at position 16 in the binding site of human E-cadherin, which is a glutamic acid residue in the mouse or rat E-cadherins (Lecuit et al., 1999). The completion of the tridimensional structure of the InlA-cadherin complex revealed that the LRR domain of InlA forms a cavity, which is filled by the N-terminal domain of the

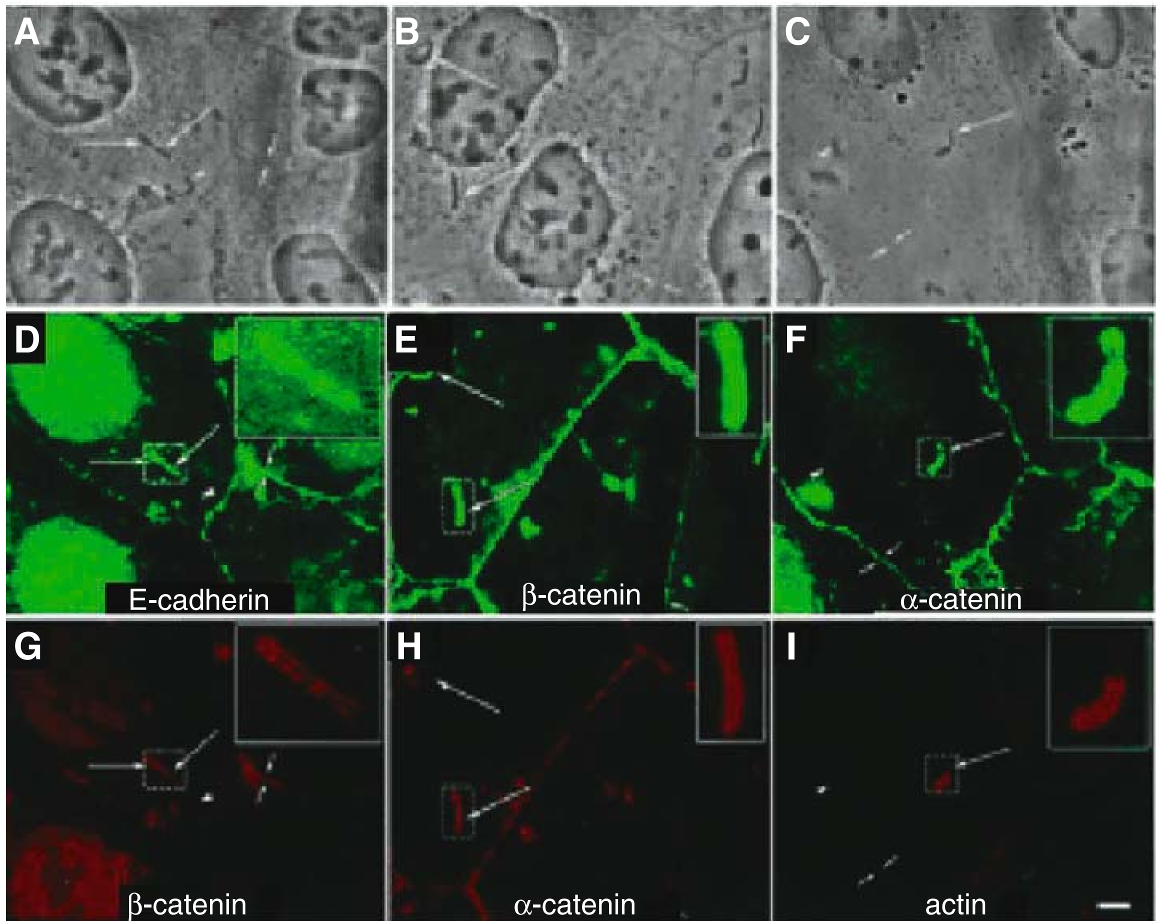

Fig. 22. Recruitment of human E-cadherin, β-catenin, α-catenin and actin during InlA-dependent entry. Caco-2 cells infected with *L. innocua* expressing InlA are observed by phase contrast (A, B and C) or by immunofluorescence microscopy after labeling of actin with fluorescent phalloidin (I) or with E- cadherin (D), β-catenin (E and G) and α-catenin (F and H) antibodies detected using secondary fluorescent antibodies. Arrows and arrowheads indicate sites where protein are or are not recruited around bacteria, respectively. Scale bars, 1 μm. From Lecuit et al. (2000), with permission.

human E-cadherin (Fig. 25). Access of E-cadherin to this pocket is blocked when the proline 16 is changed to a glutamic acid (Fig. 26), promoting a steric hindrance, which abolishes the interaction between the two molecules (Schubert et al., 2002).

Role of InlA in Virulence Before the discovery of the species specificity of the E-cadherin-internalin interaction, no role in virulence could be demonstrated for InlA using the mouse model (Gaillard et al., 1996; Gregory et al., 1996a; Pron et al., 1998). The recent demonstration of the specificity of InlA for human E-cadherin explains these data and has allowed further studies. The role of InlA was assessed in a transgenic mouse model, in which the human E-cadherin is expressed at the intestinal level. Infection of these transgenic mice with a wild-type or a Δ*inlA* mutant showed that InlA was important for the crossing of the intestinal barrier (Lecuit et al., 2001b). In guinea pigs, which possess a permissive E-cadherin, infection of the liver, spleen, lymph nodes and small intestine was strongly reduced after oral inoculation with a Δ*inlA* mutant as compared with wild-type *L. monocytogenes* (Lecuit et al., 2001b). These results confirm that, in permissive species, InlA plays a role in the crossing of the intestinal barrier. Recently, a comparative study between *L. monocytogenes* isolates from sporadic or epidemic cases or associated with healthy human carriages revealed that a high proportion of isolates from food expressed a truncated InlA (35%), whereas expression of a truncated internalin is rare in clinical isolates (7%) and notably absent in isolates from maternofetal infections (Jacquet et al., 2004). Interestingly, these isolates with truncated InlA were affected in their invasive ability, as well as in their pathogenic potential in the chick embryo model, suggesting a role for InlA in the establishment of listeriosis

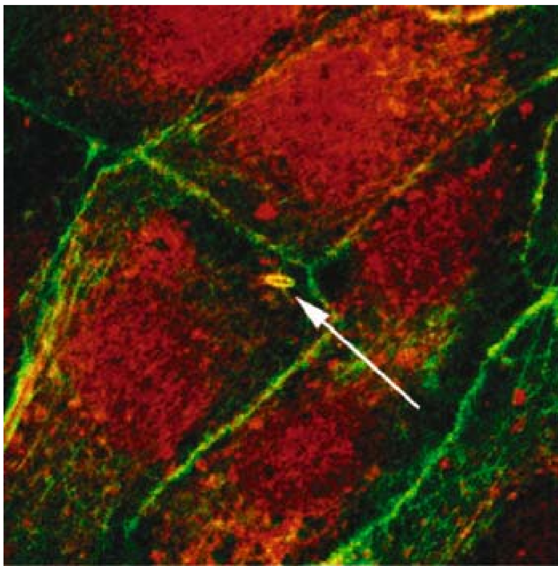

Fig. 23. Recruitment of unconventional myosin VIIa (red) and actin (green) during InlA-dependent entry. Immunofluorescence microscopy images of Caco-2 cells infected with *L. innocua* expressing InlA. Actin is labeled with fluorescent phalloidin. Unconventional myosin VIIa is labeled with specific antibodies detected with secondary fluorescent antibodies. Myosin VIIa is present at the adherens junctions of epithelial cells and is also recruited with actin at the *L. monocytogenes* entry site. Courtesy of Sandra Sousa, unpublished image.

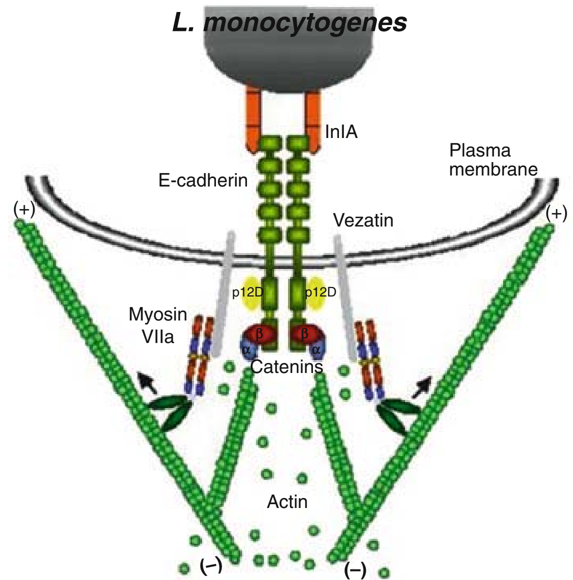

Fig. 24. Model for InlA-dependent entry of *L. monocytogenes* into epithelial cells. Proteins involved in entry are E-cadherin, α- and β-catenins, vezatin, myosin VIIa, and actin. Adapted from Sousa et al. (2004), with permission.

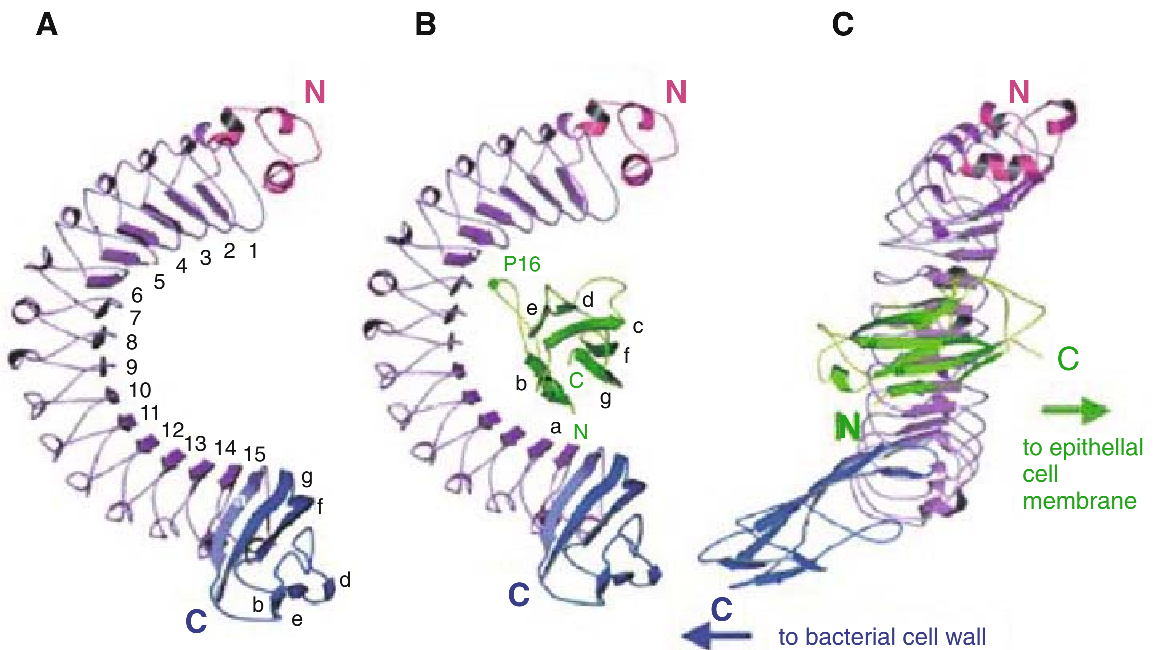

Fig. 25. Model of the three-dimensional structure of InlA' complexed or not with human E-cadherin. A) Uncomplexed InlA. B) InlA' in complex with the EC1 Ig-like extracellular domain of human E-cadherin. C) 90° complex viewed in (B) rotated 90°. InlA' is the functional domain of InlA (residues 36–496). The cap, leucine-rich repeat, and Ig-like inter-repeat domains of InlA' are represented in pink, purple and blue, respectively. The EC1 domain of human E-cadherin containing the crucial proline 16 required for efficient recognition is depicted in green. From Schubert et al. (2002), with permission.

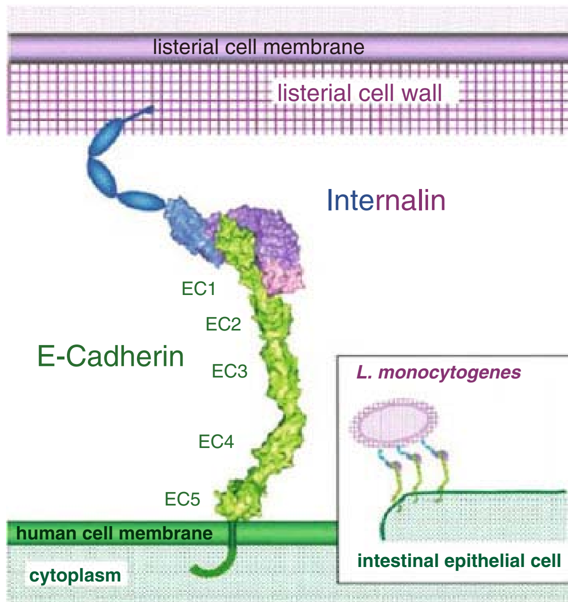

Fig. 26. Representation of the InlA-human E-cadherin complex. InlA, covalently bound to the cell wall of *L. monocytogenes*, binds to the human E-cadherin. EC1 to EC5 are the extracellular domains of human E-cadherin. The cap, leucine-rich repeat and Ig-like inter-repeat domains of InlA' are represented in pink, purple and blue, respectively. From Schubert et al. (2002), with permission.

(Jonquieres et al., 1998; Olier et al., 2003). Exchange of the regions encoding InlA between strains expressing full length or truncated InlA confirmed the requirement for InlA in entry (Olier et al., 2005). The role of InlA in *L. monocytogenes* fetoplacental tropism has now been experimentally addressed. The results show that the ability of *L. monocytogenes* to target the placental villi and cross the placental barrier is dependent upon internalin interaction with trophoblast E-cadherin (Lecuit et al., 2004). Thus, *L. monocytogenes* deploys a common strategy to target and cross the intestinal and placental barriers. This raises the possibility that *L. monocytogenes* placental tropism may be a consequence of its evolved mechanism for targeting the intestinal epithelium. Interestingly, the blood-brain barrier is composed of microvascular endothelium and choroid plexus epithelium expressing E-cadherin. Thus, it is tempting to speculate that *L. monocytogenes* targeting to and invasion of the CNS may also be mediated by the interaction between InlA and E-cadherin.

Interactions of InlB with its Receptors The *inlB* gene was first identified as the second gene of the *inlAB* operon involved in *L. monocytogenes* invasion of epithelial cells (Gaillard et al., 1991). InlB has a role in virulence predominantly for hepatic colonization, as evaluated in the murine model after intravenous infection (Gaillard et al., 1996; Dramsi et al., 1997). InlB is the major protein required for invasion in a variety of cell types in which InlA plays no role, such as hepatocytes, endothelial cells and fibroblasts (Dramsi et al., 1995; Lingnau et al., 1995; Gregory et al., 1997; Greiffenberg et al., 1998; Parida et al., 1998). InlB is sufficient to promote entry of noninvasive bacteria or beads into nonphagocytic cells (Braun et al., 1998). Soluble InlB induces important cytoskeletal rearrangements (Ireton et al., 1999), generating cell membrane ruffling or cell scattering (Shen et al., 2000). These observations support the hypothesis that InlB, when released from the bacterial surface, may facilitate the disruption of epithelial barriers, allowing dissemination of *L. monocytogenes* to deep tissues. Soluble InlB also stimulates the phagocytosis of noninvasive bacteria, probably as a consequence of cell membrane ruffling (Braun et al., 1998).

Structure and Cell Wall Association of InlB As a member of the internalin multigenic family, InlB possesses a LRR domain and a series of repeats starting with a GW dipeptide at its C-terminus. Both the LRR domain and the GW modules are involved in cell receptor recognition (Braun et al., 2000; Shen et al., 2000; Jonquieres et al., 2001; Marino et al., 2002; Machner et al., 2003). The GW modules of InlB mediate its loose association with the bacterial cell wall through interaction with the membrane-associated LTAs (Braun et al., 1997; Jonquieres et al., 2001) (Fig. 27). However, InlB can also be released in the medium and act as a soluble molecule (Jonquieres et al., 1999). Dissection of the tridimensional structure of the different domains of InlB and of the entire molecule revealed that InlB exhibits an elongated and curved structure, facilitating multiple protein-protein interactions. In addition, the X-ray structure revealed that calcium ions are bound to the N-terminal part of InlB (Fig. 28). Interestingly, the 80-amino acid long GW modules of InlB resemble SH3 domains, although the homology appears rather structural than functional (Marino et al., 1999; Marino et al., 2002).

Interactions of InlB with Its Receptors and Consequences InlB interacts with several cellular receptors, gClqR, Met and GAGs, which probably cooperate to promote bacterial uptake, but their respective roles in the signaling responses to InlB remain to be clarified. The first InlB receptor was identified by affinity chromatography as gC1qR (Braun et al., 2000). gC1qR was initially identified as the receptor for C1q, the first component of the complement cascade

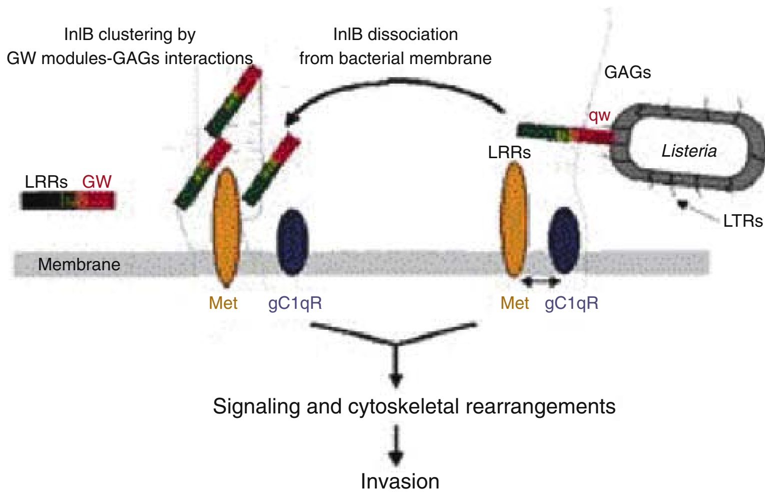

Fig. 27. Model illustrating the synergy between the N- and C-terminal domains of InlB to induce efficient signaling. The GW modules of the C-terminal domain are responsible for the loose association of InlB with the bacterial cell wall lipoteichoic acids (LTAs) and bind to the glycosaminoglycans (GAGs). This interaction can detach InlB from the bacterial surface and allow the interaction of its N-terminal leucine-rich repeat (LRRs) domain with Met, the hepatocyte growth factor receptor. From Jonquieres et al. (2001), with permission.

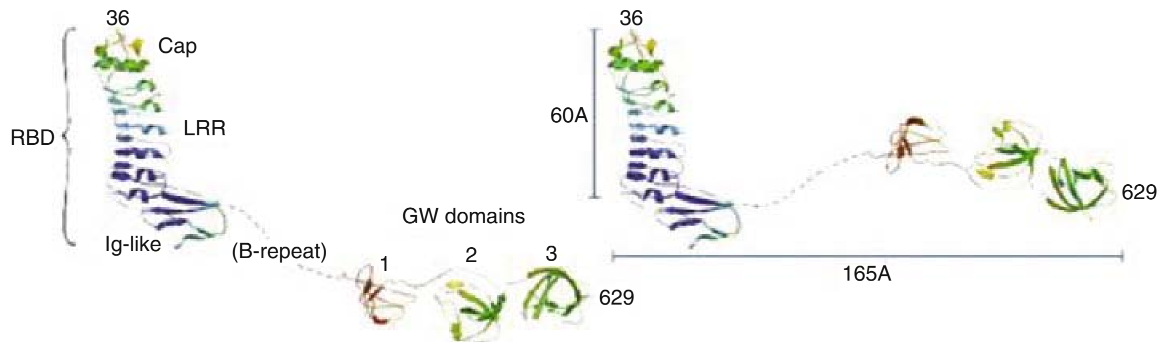

Fig. 28. Three-dimensional structure of InlB. Two different conformers of InlB differing in the path of the B-repeats are represented as ribbons. The red dotted lines represent the B-repeats (not modeled). From Marino et al. (2002), with permission.

(Peerschke et al., 1994). gC1qR is a ubiquitous protein, also called p32. It is present in different subcellular locations and has been involved in various cellular processes, including inflammation and immunity (Ghebrehiwet et al., 2001). InlB-dependent entry is blocked by anti-gC1qR antibodies and by C1q. Moreover, transfection with human gC1qR enhances cell invasion (Braun et al., 2000). These results suggest that gC1qR is an important mediator of bacterial invasion, but since it has neither a transmembrane domain nor a GPI-anchored domain, it was concluded that gC1qR would behave as a co-receptor for a signaling protein. This signaling receptor was later identified as Met, the receptor for the hepatocyte growth factor (HGF). Indeed, Met is a transmembrane protein with a tyrosine kinase activity, which mediates several signaling pathways triggered by InlB. Cells expressing little or no Met are not permissive for InlB-mediated entry and signaling but become permissive when transfected with the human Met receptor (Shen et al., 2000). Both InlB and HGF induce the clathrin-dependent endocytosis and lysosomal degradation of Met (Li et al., 2005; Veiga and Cossart, 2005). The Cbl ubiquitin ligase monoubiquitinates Met and is critical for *Listeria* entry into cells (Veiga and Cossart, 2005). InlB also binds directly to cellular GAGs through its GW modules. InlB-dependent entry into epithelial cells is strongly affected by depletion of the cellular plasma membrane GAGs. Finally, heparin, which is a GAG, promotes the detachment of InlB from the bacterial surface and its clustering, suggesting the following model for InlB interaction with its different receptors. Interaction of InlB with GAGs through its GW modules leads to its detachment from the bacterial surface, allowing its clustering at the cellular surface through binding to Met by its LRR domain and favoring the local activation of the signaling pathway downstream of Met (Jonquieres et al., 2001).

Regions of InlB Involved in Receptor Recognition It was shown that the GW modules of InlB interact with gClqR and GAGs and the LRR domain of InlB binds to Met (Jonquieres

et al., 2001; Marino et al., 2002; Shen et al., 2000). Both regins of InlB, the GW modules and the LRR domain, cooperate for Met activation and InlB-dependent entry (Banerjee et al., 2004).

Species Specificity of InlB As for InlA, InlB was shown to be species specific. It is able to promote entry and ruffling through its Met interaction in humans and mouse cells, but not in guinea pig and rabbit cells, as a problable consequence of Met differences between species (Khelef et al., in press).

InlB-Induced Activation of Phosphoinositide 3-Kinase Cell stimulation with InlB, either soluble or expressed by bacteria, promotes activation of the phosphoinositide 3-kinase (PI3K) (Ireton et al., 1996). This activity strictly depends on the activation of Met. Met recruits and phosphorylates the ubiquitin ligase, Cbl, and the adaptor molecules, Gab1 and Shc, allowing the recruitment of the p85 subunit of type I PI3K to the plasma membrane (Ireton et al., 1999; Shen et al., 2000; Sun et al., 2005). The formation of Gabl-p85 and Gabl-Crk complexes induced by InlB was shown to be important for bacterial entry (Sun et al., 2005). PI3K converts the phosphoinositide PIP2 (phosphatidylinositol 4,5-bisphosphate) into the potent second messenger PIP3 (phosphatidylinositol 3,4,5-trisphosphate), which controls several signaling pathways. In the case of InlB, stimulation of the PI3K through Met activation is critical for phagocytosis, ruffling, and cell scattering, as shown by the inhibitory effects of wortmannin and LY294002, two PI3K inhibitors (Ireton et al., 1996; Ireton et al., 1999; Shen et al., 2000). However, PI3K activation was shown to be dispensable for InlB- or HGF-induced internalization of Met but essential for its lysosomal degradation (Li et al., 2005).

InlB-Induced Phagocytosis Several regulators of actin polymerization and depolymerization are involved in the cytoskeletal rearrangements generated by the interactions of InlB with its receptors have been identified (Bierne and Cossart, 2002a). WASP-related proteins, Abi1, Ena/VASP (Bierne et al., 2005) and the actin nucleator Arp2/3 (Bierne et al., 2001) are recruited to InlB-induced phagocytic cups and membrane ruffles and are required for efficient phagocytosis (Fig. 29). Completion of phagocytosis requires the action of cofilin, which locally depolymerizes actin, allowing the retraction of the cup. Cofilin is recruited to the InlB entry site and accumulates transiently around the nascent phagosome (Fig. 29). The importance of this function was demonstrated by inactivating cofilin, by maintaining it in its inactive form, or by either overexpressing a constitutively activated cofilin mutant or using a dominant negative mutant of the LIM kinase, which inactivates cofilin. The recruitment of Arp2/3, cofilin, and LIM kinase to the entry site is proposed to occur through the activation of the small GTPase Rac and Cdc42 depending on the cell type (Bierne et al., 2001; Bierne et al., 2005).

InlB-Induced Signaling In addition to its role in phagocytosis, InlB also stimulates other PI3K-dependent pathways, such as the PLC-γ (Bierne et al., 2000), the Ras-Akt-NF-κ B signaling cascade (Mansell et al., 2000; Mansell et al., 2001), and the Ras-MAP kinases pathway (Copp et al., 2003) (Fig. 30). These different pathways have been involved in the regulation of cell survival and anti-apoptosis by growth factors, suggesting that InlB might induce these activities to counteract the deleterious effect of *L. monocytogenes* infection. Since cell membrane ruffling and scattering are also observed after stimulation with InlB and HGF, revealing that InlB behaves as a growth factor (Bierne and Cossart, 2002a).

INTERNALINS IN OTHER *LISTERIA* SPECIES Five internalin genes were first identified in *L. ivanovii* strains, but DNA hybridization experiments showed that internalin-like genes were also present in other *Listeria* species (Gaillard et al., 1991; Dramsi et al., 1997). *Listeria ivanovii* internalins harbor the typical LRR domain. They are small-secreted proteins, while most *L. monocytogenes* internalins are associated with the bacterial cell wall (Engelbrecht et al., 1998b; Vazquez-Boland et al., 2001). Importantly, the two major proteins involved in invasion by the human pathogen *L. monocytogenes*, InlA and InlB, are absent from the animal pathogen *L. ivanovii* suggesting that internalins are critical for Listeriae interactions with their hosts and environment.

Listeriolysin

Listeriolysin O or LLO was the first, and so far the most potent, virulence factor identified in *L. monocytogenes* (Gaillard et al., 1986) (Fig. 4). LLO is a 60-kDa hemolysin belonging to a family of antigenically related cholesterol-dependent pore-forming toxins or cholesterol-dependent cytolysins, which also includes the streptolysin O (SLO) of *Streptococcus pyogenes* and perfringolysin O (PFO) of *Clostridium perfringens* (Gilbert, 2002). Cholesterol-dependent cytolysins are inhibited by oxidation or by thiol-reacting products but reactivated by thiol-reducing compounds (Alouf, 1999). They are characterized by a ECTGLAWEWWR motif, which includes the

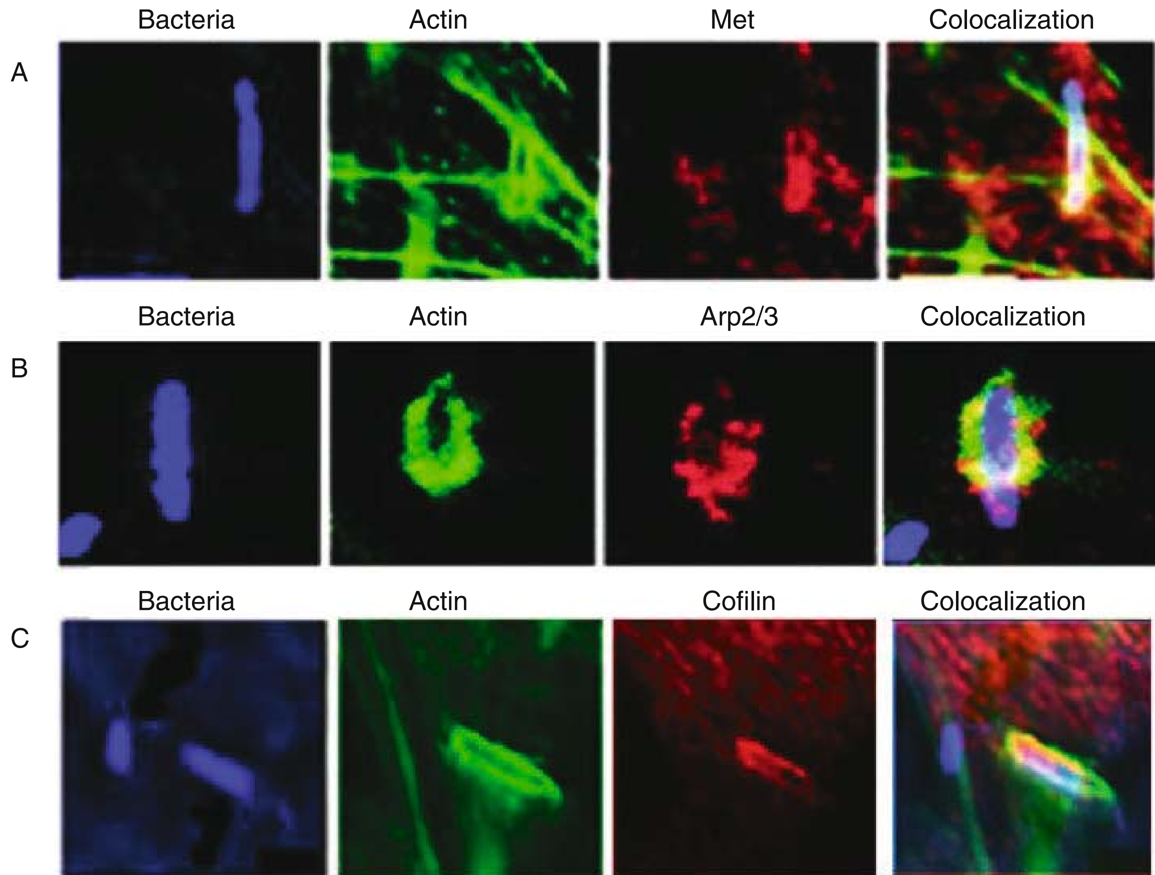

Fig. 29. Recruitment of Met (hepatocyte growth factor receptor), Arp2/3 complex, cofilin and actin during InlB-dependent entry. Immunofluorescence microscopy images of Vero (A and B) or Ref52 (C) cells infected with *L. monocytogenes*. Bacteria appear in blue. Actin is labeled with fluorescent phalloidin (green). Met, Arp2/3 and cofilin are labeled with specific antibodies detected with secondary fluorescent antibodies (red). Colocalization corresponds to overlays of green and red images. From Bierne et al. (2001), with permission.

unique cysteine residue of the LLO. However, mutation of this residue is not essential for hemolysis, while the last Trp residue of the motif (Trp-432) was shown to play a crucial role for full hemolytic activity and virulence (Michel et al., 1990; Gilbert, 2002).

LLO is encoded by the *hly* gene, which was first identified as a factor absolutely required for virulence by analyzing nonhemolytic mutants obtained by transposon mutagenesis (Gaillard et al., 1986; Kathariou et al., 1987). Virulence of the nonhemolytic mutants is strongly attenuated in the mouse model, with a four log difference in the LD_{50} after intravenous inoculation (Gaillard et al., 1986; Kathariou et al., 1987). Nonhemolytic mutants are also affected in both epithelial and phagocytic cellular models of infection, in which they fail to escape from the phagosomes and to multiply intracellularly (Berche et al., 1988; Kuhn et al., 1988). Genetic analysis of these mutants showed that the LLO defect could be complemented by the reintroduction in trans of the *hly* gene (Cossart et al., 1989b). Similarly, spontaneous *hly* revertants recover their hemolytic and pathogenic properties (Gaillard et al., 1986), confirming the central role of LLO in these processes.

LLO is required for escape from both the primary vacuole and of the double membrane-bound phagosomes, cooperating with the PLC-A and PLC-B. It is essential for intracellular multiplication (Gaillard et al., 1987; Bielecki et al., 1990; Gedde et al., 2000). It is proposed that the pore forming ability of LLO mediate the lysis of the vacuoles. In addition, LLO is also responsible for important features of *L. monocytogenes* interactions with the host. They include activation of several signaling pathways (Wadsworth and Goldfine, 1999; Repp et al., 2002; Wadsworth and Goldfine, 2002; Dramsi et al., 2003), production of cytokines (Nishibori et al., 1996), induction of apoptosis of dendritic cells and lymphocytes in vitro (Guzman et al., 1996; Carrero et al., 2004) and of lymphocytes during mice infection (Carrero et al., 2004), and development of a protective immune response against *L. monocytogenes* infection (Berche et al., 1987a;

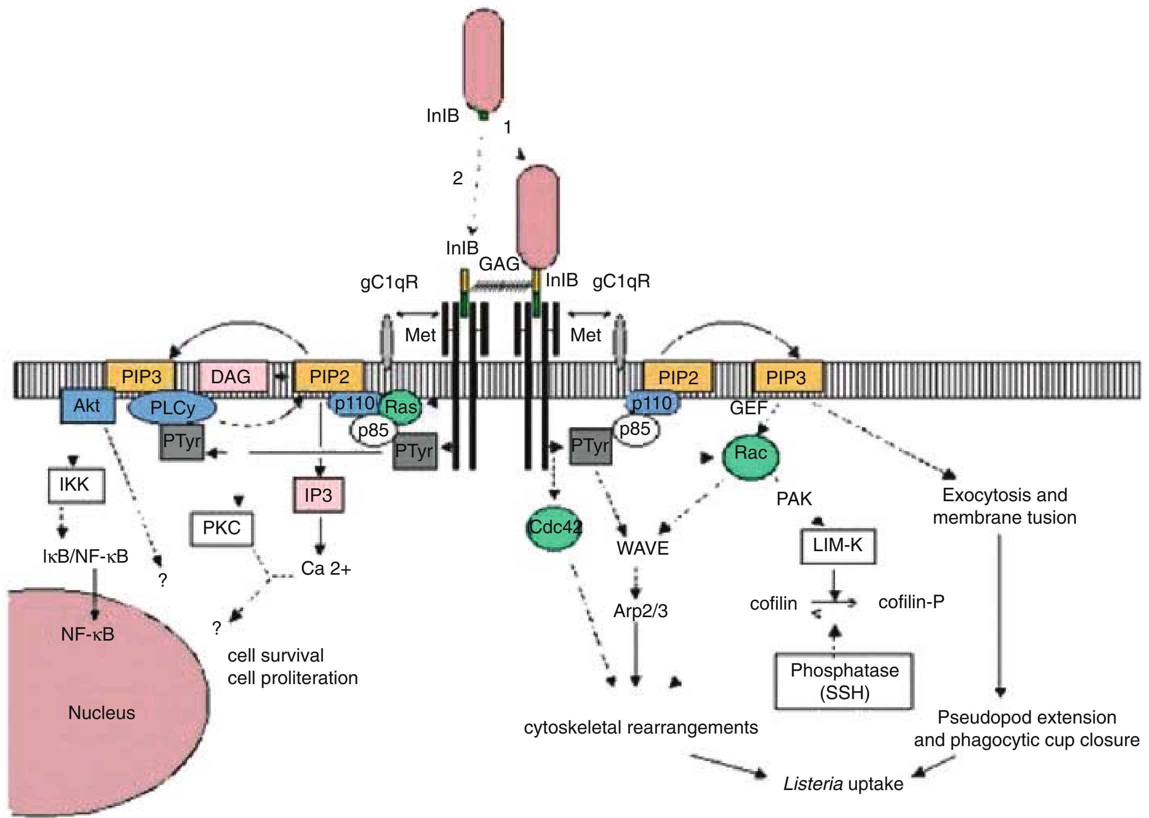

Fig. 30. The InlB-mediated signaling pathway. InlB buried in the bacterial cell wall may dissociate from the bacterial surface by interacting with glycosaminoglycans (GAGs) and possibly gC1qR (C1q complement component receptor), becoming accessible to bind Met (hepatocyte growth factor receptor). The surface-exposed InlB will trigger entry of the bacteria (1) while a pool of soluble InlB released in the medium will signal independently or as a prelude to bacterial entry (2). Interaction of InlB with Met induces the recruitment of adaptor proteins, the recruitment and activation of the phosphoinositide 3-kinase (PI3K). This triggers actin cytoskeletal rearrangements involving Rho-GTPases and cytoskeletal regulatory proteins and membrane reorganization, which lead to bacterial uptake. Activation of other signaling molecules such as phospholipase C γ (PLC-γ) and the kinase Akt might affect the fate of bacteria and/or cells. From Bierne and Cossart (2002a), with permission.

Pamer, 2004). Antibodies specific for LLO are produced during human listeriosis and their detection is used for diagnosis (Berche et al., 1990). Interestingly, because it can lyse phagosomal membranes, LLO (alone or included in liposomes) has been used as an intracytosolic delivery system for antigens, DNA, or antisense oligodeoxynucleotides. This strategy has been exploited to facilitate research progress in gene therapy, cancer treatment, and vaccination (Lee et al., 1996; Dietrich et al., 2001; Mathew et al., 2003; Provoda et al., 2003).

Role of Listeriolysin The pathogenic potential of Listeriae has been linked to their ability to induce hemolysis, and therefore to the production of LLO. However, despite its fundamental role in virulence, LLO by itself is not sufficient to confer a pathogenic potential to nonpathogenic Gram-positive bacteria (Portnoy et al., 1992). Moreover, *L. seeligeri*, which has weak hemolytic activity, is rarely pathogenic for humans, suggesting that hemolysin is not the only factor required for *L. monocytogenes* virulence (Seeliger and Jones, 1986; Hof and Hefner, 1988). Heterologous expression of the *hly* gene in *Bacillus subtilis* enabled this organism to lyse primary phagosomes and to multiply intracellularly, confirming the role of LLO escaping from the primary vacuole (Bielecki et al., 1990). When this experiment was performed with the genes encoding PFO or SLO, it was found that only PFO, and not SLO, promoted phagosomal disruption (Portnoy et al., 1992). This shows that despite the high level of homology of Cholesterol-dependent cytolysins, their ability to disrupt the phagosomal membrane is not conserved. Similarly, expression of ivanolysin, the LLO homologue expressed by *L. ivanovii*, can replace LLO to allow vacuolar escape of *L. monocytogenes*. However, it is not sufficient to confer full virulence in mice, as a •*hly L. monocytogenes* mutant expressing ivanolysin colonizes

efficiently the liver but does not persist in the spleen (Frehel et al., 2003).

MECHANISMS UNDERLYING LISTERIOLYSIN ACTIVITY On the basis of structure-function and biochemical studies, as well as the sequence homology between LLO, PFO and SLO, the mechanism of LLO interaction with membranes is currently thought to be as follows. After an initial step of binding to membrane cholesterol, the toxin monomers diffuse laterally to form ring-shaped oligomers inserted in the membrane bilayer, forming ion-permeable pores (30 nm in diameter) without disrupting the plasma membrane (Vazquez-Boland et al., 2001; Repp et al., 2002) (Fig. 31). Optimal hemolytic activity of LLO is observed at acidic pH, as in the case of phagosomes containing *L. monocytogenes*, supporting the idea that LLO is activated inside the phagosome and inactivated in the cytosol, preventing cellular damage (Geoffroy et al., 1987; De Chastellier and Berche, 1994; Beauregard et al., 1997). Interestingly, LLO is degraded at neutral pH, as a consequence of its denaturation, which results from the unfolding of its transmembrane domain. This process also contributes to the control of LLO activity (Schuerch et al., 2005). Permeabilization of the vacuolar membrane occurs as the pH rises, probably resulting from the equilibration with the cytosol (Beauregard et al., 1997). Experimental evidence for the role of LLO in this process was provided using a mutant of *L. monocytogenes* carrying an IPTG-inducible *hly* gene. IPTG induction was performed when bacteria were in the phagosome, resulting in production of active LLO and disruption of the phagosomal membrane that promoted bacterial release in the cytoplasm (Dancz et al., 2002). Disruption of the primary phagosome and of the double membrane vacuole requires the concomitant effect of the LLO and phospholipase activities (PLC-A and PLC-B, respectively) and leads to disruption of the phagosome, releasing *L. monocytogenes* into the cytosol (Beauregard et al., 1997; Glomski et al., 2002). Interestingly, LLO is not always essential for phagosome disruption, as in certain cell types (HeLa and HenLe 407), the lysis of the vacuole occurs in absence of LLO and is only mediated by PLC-B (Marquis et al., 1995; Grundling et al., 2003).

Interactions of Listeriolysin with Cholesterol Cholesterol is an important factor for LLO binding to plasma membrane. However, preincubation of LLO with cholesterol inactivates its hemolytic activity without blocking its binding to plasma membranes (Geoffroy et al., 1987; Jacobs et al., 1988; Coconnier et al., 2000). Inhibition appears to occur at the step of toxin oligomerization rather than at the initial step of membrane association (Jacobs et al., 1998; Coconnier et al., 2000). In polarized intestinal cells, LLO is specifically targeted to plasma membrane lipid rafts, probably because of its high affinity for cholesterol, and is internalized through caveolae (Coconnier et al., 2000). In agreement with this, LLO was also shown to aggregate several raft-associated molecules, including a signaling molecule, the Lyn kinase, a process which is proposed to mediate LLO signaling ability. This aggregation is independent of the cytolytic activity of LLO and results from the oligomerization of LLO monomers in the membrane (Gekara et al., 2005). In polarized intestinal cells, internalization of LLO through caveolae leads to stimulation of mucus exocytosis in cultured intestinal mucus secreting cells (Coconnier et al., 2000).

pH Dependence of Listeriolysin As compared with other Cholesterol-dependent cytolysins, the main feature of LLO is its optimal activity at acidic pH (Geoffroy et al., 1987). This property has important consequences since LLO is fully active in acidic vacuoles, which allows escape from the phagosome without destroying the plasma membrane (Beauregard et al., 1997). An elegant study has shown that this property is

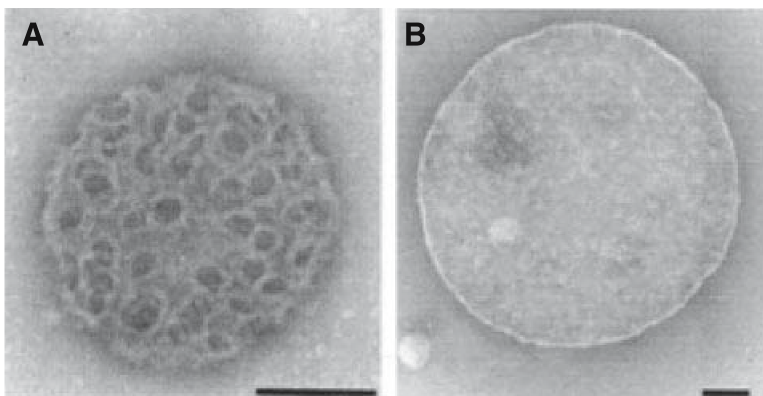

Fig. 31. Electron micrograph of red blood cells after treatment with listeriolysin (A) or cholesterol-inactivated listeriolysin (B). Scale bars, 100 nm. From Jacobs et al. (1998), with permission.

important for completion of *L. monocytogenes* infectious cycle. Indeed, intracellular expression of PFO under the control of the *hly* promoter resulted in lysis of the phagosomal membrane but was followed by cell lysis. Mutations in PFO modifying its optimal pH (from neutral to acidic) abolished its cytotoxicity without affecting its ability to disrupt the phagosome (Jones et al., 1996). Conversely, mutations in LLO modifying its pH optimum (from acidic to neutral) conferred a stronger hemolytic activity but also a lower pathogenic potential. Indeed, a *L. monocytogenes* mutant with a LLO active at neutral pH was able to lyse the primary phagosome and to disseminate by cell-to-cell spreading. It was more cytolytic than the wild-type strain. Its LD_{50} was 100-fold higher in the mouse model (Glomski et al., 2002). This attenuated virulence is probably due to the release of cytolytic bacteria into the extracellular milieu and their exposure to antimicrobial defenses. Similarly, mutants of LLO that fail to compartmentalize its activity were more cytotoxic in vitro but less virulent in vivo (Glomski et al., 2003).

PEST-like Motif of Listeriolysin A recent analysis of the N-terminal domain of LLO revealed the presence of a PEST-like sequence, which is involved in targeting proteins to the proteasome dependent degradation pathway. LLO mutants lacking this sequence were cytotoxic and were affected in virulence (Decatur and Portnoy, 2000; Lety et al., 2001). The presence of this PEST-like motif could appear as another strategy to overcome the toxic potential of LLO by targeting the toxin to the degradation pathway when released in the cytosol. However, this theory is challenged by controversial results concerning the role of the PEST motif. One group showed that deletion of the PEST sequence resulted in LLO accumulation in the cytosol without defect in escape from the phagosome (Decatur et al., 2001). Another group showed that mutations in or near the PEST motif of LLO did not promote accumulation of the toxin in the cytosol but decreased LLO ability to disrupt the phagosome. This suggests that the conformation of the domain surrounding the PEST motif, rather than the PEST sequence itself, is important for this activity (Lety et al., 2002; Lety et al., 2003).

LISTERIOLYSIN-INDUCED SIGNALING IN EUKARYOTIC CELLS LLO is a very potent inducer of cell signaling involved in several biological processes, ranging from crucial features of the infectious process to other signals whose roles remain to be clarified. These include stress responses, cell proliferation, secretion of mucus, immunomodulation, or activation of proinflammatory cascades.

Activation of Second Messengers In epithelial and endothelial cells, the pore forming ability of LLO stimulates a transient influx of extracellular calcium, which is mediated by its pore forming ability (Wadsworth et al., 1999; Rose et al., 2001; Repp et al., 2002; Dramsi et al., 2003). The consequences of this activity include modulation of cellular gene expression, signaling, and modulation of *L. monocytogenes* invasion in epithelial cells. LLO modifies the phosphoinotide metabolism and the generation of lipid mediators, such as diacylglycerol, platelet-activating factor, ceramide, and prostaglandins in endothelial cells or platelet-activating factor and leukotrienes in polymorphonuclear leukocytes (PMN) (Sibelius et al., 1996a; Sibelius et al., 1996b; Sibelius et al., 1999; Rose et al., 2001). LLO also induces PMN degranulation characterized by elastase secretion (Sibelius et al., 1999). In macrophages, LLO induces an increase in phosphoinositide hydrolysis, activation of the host PI-PLC, phospholipase D, and protein kinase C (PKC) α and β (Goldfine et al., 2000; Wadsworth and Goldfine, 2002).

Activation of MAP Kinases and Nuclear Factor Cascades LLO induces several well-known signaling cascades, including the Raf-MEK-MAP kinase pathway (Tang et al., 1996; Tang et al., 1998; Weiglein et al., 1997), the NF-κ B and the AP-1 nuclear factor pathways (Kayal et al., 2002; Lievin-Le Moal et al., 2002), as well as calcium- or lipid-dependent signaling pathways. LLO-dependent activation of MAP kinases in HeLa cells concerns Erk2, p38 MAPK, and c-Jun. Hemolytic activity is required for the activation of MAP-kinase activation and for *L. monocytogenes* invasion of HeLa cells (Tang et al., 1998). LLO-induced activation of NF-κ B is implicated in cytokine production, endothelial cell activation, and mucus secretion (Kayal et al., 1999; Rose et al., 2001; Lievin-Le Moal et al., 2002). Activation of NF-κ B involves the classical inactivation of the NF-κ B inhibitor Iκ B kinase in a process that does not involve the interleukin 1 (IL1) signaling pathway (Kayal et al., 2002).

Activation of Mucus Secreting Cells Interestingly, LLO activates the expression of mucin genes and the exocytosis of mucins by polarized intestinal mucus-secreting cells through activation of NF-κ B and AP-1 transcription factors (Lievin-Le Moal et al., 2002). This process is independent of the pore-forming activity of LLO (Coconnier et al., 2000). Importantly, induction of these activities by LLO results in the inhibition of *L. monocytogenes* entry into mucin secreting cells (Lievin-LeMoal et al., 2005).

Activation of Proinflammatory Cytokines Among the most important signaling activities mediated by LLO is its ability to induce the expression of a wide range of proinflammatory molecules in both macrophages and endothelial cells (Krull et al., 1997; Rose et al., 2001). LLO, either purified or expressed by *L. monocytogenes*, induces the production of interleukins (IL1 and IL12), tumor necrosis factor α (TNFα) and type I interferon β (IFNβ) by macrophages as well as interferon γ (IFNγ) by natural killer (NK) cells (Yoshikawa et al., 1993; Nishibori et al., 1996; Kohda et al., 2002; Nomura et al., 2002; Stockinger et al., 2002). Interestingly, both IL1 and IFNγ production are independent of the hemolytic activity of LLO (Yoshikawa et al., 1993; Nishibori et al., 1996). LLO also activates endothelial cells by stimulating the production of nitic oxide, adhesion molecules (ICAM–1, V-CAM-1 and E-selectin), chemokines and proinflammatory cytokines (MCP-1, IL6, IL8 and GM-CSF), probably through NF-κ B activation (Krull et al., 1997; Drevets, 1998; Kayal et al., 1999; Rose et al., 2001). It has been proposed that, in vivo, the production of IFNγ or TNFα mediate the endothelial cell activation, allowing the recruitment of T cells to the site of infection and contributing to the protective acquired immune response against *L. monocytogenes* (Xiong et al., 1994; Vazquez et al., 1995). In the epithelial cell line Caco-2, membrane permeabilization by LLO was shown to trigger expression of the interleukin IL6, in a Ca^{2+} dependent manner (Tsuchiya et al., 2005).

Role of Listeriolysin in the Protective Immune Response LLO is involved in the induction of a protective immune response against *L. monocytogenes* infection in different ways. LLO is a major protective antigen recognized by cytotoxic $CD8^{+}$ lymphocytes, in agreement with the cellular immune response characterizing *L. monocytogenes* infection (Berche et al., 1987a; Bouwer et al., 1992; Sirard et al., 1997). Moreover, presentation of major histocompatibility complex (MHC)-I restricted antigens of *L. monocytogenes* and induction of a specific protective immune response result from the release of bacteria inside the cytosol and their intracellular growth. Since LLO mediates this process, it is therefore important for an efficient cellular immune response against *L. monocytogenes* (Darji et al., 1995; Darji et al., 1997). However, LLO also elicits a strong humoral immune response, which, in restricted cases, can be protective against *L. monocytogenes* infection (Berche et al., 1990; Gholizadeh et al., 1996; Grenningloh et al., 1997; Edelson et al., 1999). This has led to the reconsideration of the previous thought that protective immunity against *L. monocytogenes* infection was only cellular and not humoral. Finally, LLO mediates the delivery of soluble antigen to the MHC-I presentation pathway, which is another way of participating in the induction of specific cellular immunity (Berche et al., 1987b; Brunt et al., 1990; Hitbold et al., 1996; Lee et al., 1996).

Phospholipases and Metalloprotease

Two phospholipases are produced by *L. monocytogenes*, PLC-A or PI-PLC and PLC-B or PC-PLC (Mencikova, 1989; Geoffroy et al., 1991; Leimeister-Wachter et al., 1991; Mengaud et al., 1991a; Mengaud et al., 1991c). Another phospholipase SmcL is produced by *L. ivanovii* (Mencikova, 1989; Gonzalez-Zorn et al., 1999). The three phospholipases have a membrane damaging activity and are involved in bacterial escape from primary and/or/or secondary phagosomes (Vazquez-Boland et al., 1992; Camilli et al., 1993; Marquis et al., 1995; Gonzalez-Zorn et al., 1999; Grundling et al., 2003). Each of the two *L. monocytogenes* phospholipases is important for virulence since mutants deficient in either PLC-A or PLC-B are attenuated (Camilli et al., 1991; Smith et al., 1995). More importantly, double mutants deficient in both phospholipases are 500 times less virulent than single mutants, emphasizing the importance and the complementarity of these factors in listeriosis (Smith et al., 1995).

Phospholipase PLC-A The phospholipase PLC-A is a 33-kDa secreted protein, whose enzymatic activity is specific for PI (PI-PLC) (Mengaud et al., 1991a). PLC-A is produced by *L. monocytogenes* and *L. ivanovii* and is similar to the PI-PLCs of *Bacillus thuringensis*, *Bacillus cereus* and *Staphylococcus aureus* (Leimeister-Wachter et al., 1991; Mengaud et al., 1991a). PLC-A is encoded by the *plcA* gene, which is upstream of the transcriptional activator gene *prfA* and opposite the *hly* gene encoding listeriolysin O (Leimeister-Wachter et al., 1991; Mengaud et al., 1991a; Mengaud et al., 1991b). During early exponential growth, the *plcA* and *prfA* genes are cotranscribed in a 2.2-kb plcA-prfA transcript. During late exponential growth, *plcA* gene expression is monocistronic. PLC-A expression is dependent on the transcriptional activator PrfA (Mengaud et al., 1991b). The PLC-A crystal structure revealed that it consists of a single $(\beta\alpha)_8$-barrel domain with its active site at the C-terminal side of the β-barrel (Fig. 32). This structure is highly homologous to the *B. cereus* PI-PLC despite the low sequence homology between the two proteins (Moser et al., 1997).

Enzymatic Activities of PLC-A PLC-A is specifically active on PI but not on PC, phospha-

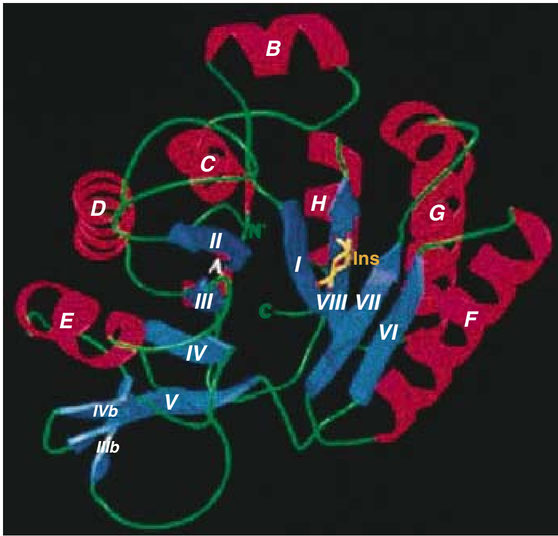

Fig. 32. Three-dimensional structure of phosphatidylinositol phospholipase C (PLC-A). Ribbon diagram of the structure of PLC-A viewing towards the active site pocket with a bound inositol molecule (Ins, bonds in yellow). α-Helices, β-strands and loops are colored in red, blue and green, respectively. From Moser et al. (1997), with permission.

tidylethanolamine or phosphatidylserine (Goldfine and Knob, 1992). It is active on eukaryotic GPI-anchored proteins including those of *Trypanosoma brucei* membranes, although with a low efficiency as compared with *B. thuringensis* PLC (Goldfine and Knob, 1992; Gandhi et al., 1993). PLC-A has a pI of 9.4 and an optimal pH of 5.5–6.5 in Triton X-100 micelles, in agreement with its intraphagosomal activity (Goldfine and Knob, 1992). Its activity is stimulated by salts, such as $CaCl_2$, $MgCl_2$ or KCl, with no specific dependence on divalent cations, and is not inhibited by EDTA (ethylene diamine tetraacetic acid) (Goldfine and Knob, 1992). Using a fluorigenic substrate analog of PI, the enzymatic properties of the PLC-A were analyzed. It was shown that a short-chain phospholipid (diC(6)PC) activates the enzyme in a process fitting with a two-site model, in which the substrate and the activator bind to different sites interacting with each other (Ryan et al., 2002). Mutations of the two potential active-site histidine residues (H38 and H86) revealed that they are both required for the enzymatic activity and for efficient escape from primary phagosomes (Bannam and Goldfine, 1999). The phospholipase activity of PLC-A can be detected on ALOA chromogenic media, on which *Listeria* expressing PLC-A appear surrounded by a green halo (AES Laboratoires).

Signaling Induced by PLC-A PLC-A mediates endothelial cell activation, characterized by the production of ceramide, activation of NF-κB, and an increased expression of E-selectin, which stimulates PMN rolling and adhesion (Schwarzer et al., 1998). In endothelial cells and in neutrophils, PLC-A also enhances the phosphoinositide metabolism and the generation of lipid mediators induced by listeriolysin O but is not able to promote this effect by itself when expressed by *L. innocua* (Sibelius et al., 1996a; Sibelius et al., 1999; Goldfine et al., 2000). In macrophages, *L. monocytogenes* infection induces a biphasic activation of NF-κ B. The first transient phase is stimulated by bacterial binding or by purified LTAs. The second persistent phase appears when the bacteria reach the cytosol and correlates with the intracellular expression of PLC-A and PLC-B (Hauf et al., 1997; Goldfine et al., 2000). In addition, PLC-A, along with LLO and PLC-B, is in part responsible for the Ca^{2+} signaling produced by *L. monocytogenes* infection. Indeed, instead of the three calcium spikes produced upon bacterial infection, a Δ*plcA* mutant generates only a single spike, with no consequence on the efficacy of entry (Wadsworth and Goldfine, 1999).

Role of PLC-A in Infection and Virulence PLC-A has a minor individual role in escape from the primary phagosome but rather acts in synergy with listeriolysin O and PLC-B to fulfill this function, as shown by using simple or double mutants (Camilli et al., 1993; Marquis et al., 1995; Smith et al., 1995). It has no role in cell-to-cell spread, suggesting no role in escape from the double membrane vacuole (Smith et al., 1995). In macrophages, *L. innocua* expressing PLC-A are able to grow inside phagosomes but do not escape from these vacuoles, while *L. innocua* fail to replicate intracellularly, suggesting that PLC-A may possess a secondary function required during intracellular growth (Schwan et al., 1994). *plcA* mutants are slightly less virulent in mice after intravenous inoculation and are defective for liver but not spleen infection (Camilli et al., 1993). However, double Δ*plcA*-Δ*plcB* mutants are much less virulent than Δ*plcB* mutants, confirming the synergy of the two phospholipases in promoting an efficient infection (Smith et al., 1995).

Phospholipase PLC-B The phospholipase PLC-B is a 29-kDa protein with phosphatidylcholine phosphohydrolase activity (PC-PLC, also named lecithinase) produced by *L. monocytogenes* and *L. ivanovii* (Ralovich et al., 1972; Geoffroy et al., 1991; Vazquez-Boland et al., 1992). PLC-B is encoded by the gene *plcB* and is expressed as an inactive precursor. It can be activated by proteolytic cleavage involving the zinc-dependent metalloprotease Mpl, encoded by the gene *mpl*, and also by cellular proteases (Geoffroy et al.,

1991; Raveneau et al., 1992; Marquis et al., 1997). The *plcB* and *mpl* genes are part of an operon, which is controlled by the transcriptional activator PrfA (Mengaud et al., 1991b). The gene *plcB* is homologous to the genes encoding the lecithinases of *Bacillus cereus* and *Clostridium perfringens* (Vazquez-Boland et al., 1992).

Production and Activation of PLC-B In vitro, the PLC-B precursor and its activator Mpl are both secreted and tightly but noncovalently associated with the bacterial cell wall (Snyder and Marquis, 2003). During intracellular growth, translocation of PLC-B is inefficient and it remains at the membrane-cell wall interface. Upon acidification of phagosomes, pools of inactive PLC-B are translocated across the bacterial cell wall (Marquis et al., 1997; Snyder and Marquis, 2003). Activation of PLC-B requires the cleavage of a propeptide. The Mpl-dependent cleavage of PLC-B correlates with its cell wall translocation (Yeung et al., 2005). However, it was shown that PLC-B activation also occurs through a Mpl-independent process mediated by a lysosomal cysteine protease (Marquis et al., 1997).

Enzymatic Activities of PLC-B PLC-B is a zinc-dependent, calcium-independent enzyme, with an optimal activity at pH 5.5–7, in agreement with its role in acidic phagosomes containing *L. monocytogenes* (Geoffroy et al., 1991; Marquis and Hager, 2000). It has a lecithinase activity and is weakly hemolytic (Geoffroy et al., 1991). PLC-B has a broad substrate spectrum and hydrolyzes several lipids, including PC, phosphatidylethanolamine or phosphatidylserine and, to a lesser extent, sphingomyelin and PI (Geoffroy et al., 1991; Goldfine et al., 1993).

Signaling Induced by PLC-B Like PLC-A, PLC-B induces endothelial cell activation characterized by the production of ceramide, activation of NF-κ B, and an increased expression of E-selectin, which stimulates PMN rolling and adhesion, an event which might be important during systemic listeriosis (Schwarzer et al., 1998). Similarly, PLC-B mediates the persistent phase of NF-κ B activation in macrophages when delivered into the cytoplasm during *L. monocytogenes* infection (Hauf et al., 1997). In addition, PLC-B, along with LLO and PLC-B, mediates the Ca^{2+} signaling produced by *L. monocytogenes* infection, which is normally characterized by three calcium spikes. Instead, a Δ*plcB* mutant generates only the first spike resulting in a delayed bacterial internalization rate (Wadsworth and Goldfine, 1999).

Role of PLC-B in Infection and Virulence PLC-B deficient mutants, generated by deletion of the *plcB* or *mpl* gene, are less virulent in mice after intravenous injection (Raveneau et al., 1992). PLC-B also plays a crucial role during murine cerebral listeriosis, since Δ*plcB* mutants are strongly attenuated in the intracranial model of infection (Schluter et al., 1998). PLC-B is involved in the lysis of the double membrane vacuole and therefore in cell-to-cell spreading, as shown with a Δ*plcB* mutant, which forms small plaques on fibroblast cultures (Vazquez-Boland et al., 1992). In certain cell types, such as HenLe 407 and HeLa, but not in macrophages, PLC-B lyses the membrane of primary phagosomes containing *L. monocytogenes* in the absence of listeriolysin (Gaillard et al., 1987; Bielecki et al., 1990; Marquis et al., 1995; Grundling et al., 2003). This suggests that depending on the cell type, PLC-B may act alone or only help LLO to lyse the primary vacuole.

The Sphingomyelinase SmcL of Listeria ivanovii The sphingomyelinase SmcL is produced by the ruminant pathogen *L. ivanovii*. It is encoded by the gene *smcL*, which is present only in *L. ivanovii* but is highly homologous to the sphingomyelinase of *Staphylococcus aureus*, *Bacillus cereus* and *Leptospira interrogans*. SmcL expression is not controlled by the transcriptional regulator PrfA. Its enzymatic activity is responsible for the bizonal hemolysis of *L. ivanovii* and for the CAMP-like reaction, which corresponds to the synergistic hemolysis of *Rhodococcus equi* and *L. ivanovii* (Figs. 4 and 5). SmcL is required for virulence in mice and has a membrane damaging activity, which is required for vacuolar escape of *L. ivanovii* and its intracellular growth (Gonzalez-Zorn et al., 1999). The crystal structure of SmcL has been solved at a 1.9 angstroms resolution, revealing that SmcL adopts a DNaseI-like fold and may bind to phospholipids (Openshaw et al., 2005).

ActA ActA is the protein that mediates actin-based motility (Cossart and Bierne, 2001; Frischknecht and Way, 2001). ActA plays a key role in intracellular movements of *L. monocytogenes*, cell-to-cell spread, and consequently bacterial dissemination into host tissues (Kocks et al., 1992). ActA mutants grow as microcolonies in infected cells (Fig. 33) and their virulence is strongly affected (Domann et al., 1992; Brundage et al., 1993). ActA has also been reported to be required for *L. monocytogenes* entry into epithelial cells (Suarez et al., 2001) and for the recognition of the heparan sulfate receptor at the cell surface (Alvarez-Dominguez et al., 1997).

ActA is a surface protein, which is 610 amino acids long after cleavage of the signal peptide (Domann et al., 1992; Kocks et al., 1992). Note that articles differ in the way they number posi-

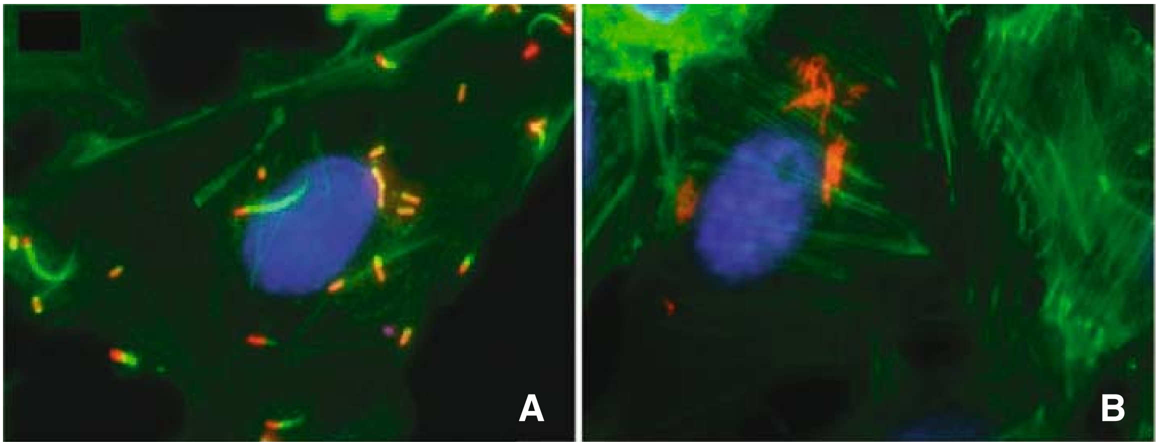

Fig. 33. Microcolonies formed by an ActA mutant in Vero cells. After infection for 6 h with *L. monocytogenes* wild-type (A) or ActA mutant (B), Vero cells were fixed. Actin was stained with rhodamine phalloidin (red) or with an anti-*L. monocytogenes* antibody. The ActA mutant, unable to move intracellularly, develops as microcolonies (arrows in B).

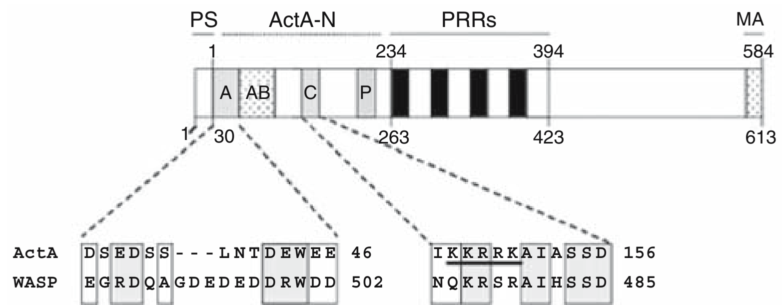

Fig. 34. ActA structure and critical regions. Schematic representation of ActA domain structure and sequence alignment with WASP (Wiskott-Aldrich syndrome protein). A, acidic domain; AB, actin-binding site; C, cofilin homology domain; MA, membrane anchor; P, PI-binding site; PRR, proline-rich repeats; PS, peptide signal. From Cossart and Bierne (2001), with permission.

tions in the sequence, the codon 1 being either the first codon of the gene (Kocks et al., 1992) or that of the mature protein (Domann et al., 1992).

ActA is anchored to the bacterial membrane by its C-terminal hydrophobic region. Interestingly this region also allows anchoring of heterologous proteins on the mitochondrial surface when these proteins are expressed in eukaryotic cells (Pistor et al., 1994; Friederich et al., 1995). The ActA protein comprises a highly charged N-terminal domain (amino-acids 1–233), a central part made of four proline-rich repeats (amino acids 234–394) and a C-terminal part (amino acids 395–584) (Fig. 34). The ActA protein is a dimer, as originally shown by a yeast two-hybrid approach (Mourrain et al., 1997). In mammalian cells, it is phosphorylated, but the role of this phosphorylation, if any, has not been addressed (Brundage et al., 1993). ActA has been shown to bind phosphoinositides in vitro (Cicchetti et al., 1999; Steffen et al., 2000), but this property has not been investigated in detail. ActA could titrate PIP2, a phosphoinositide that controls the activity of some actin-binding proteins such as capping protein (Steffen et al., 2000).

ActA is polarly distributed on the bacterial surface (Fig. 35). Efforts to understand the origin of this polar distribution have led to the conclusion that the protein accumulates on the two old poles during growth and before division and is not incorporated at the septum during cell division (Kocks et al., 1993). After division, ActA is thus essentially located at one pole and absent from the new pole (Fig. 35). Interestingly, although the polar distribution of ActA, when discovered, appeared quite striking in view of its function in the formation of a polar actin tail (Smith et al., 1995), it has now been shown that many other, but not all, listerial surface proteins display a similar distribution. InlA, for example, is polarly distributed on the bacterial surface (Lebrun et al., 1996), but the amidase Ami does not seem to be polarly distributed (Braun et al., 1997).

ActA is necessary and sufficient to promote actin polymerization. This was first shown by expression of the *actA* gene after transfection in mammalian cells (Pistor et al., 1994). In this experiment, the ActA protein induced actin polymerization and aberrant membrane deformation when targeted to the inner face of the plasma membrane (Friederich et al., 1995). The critical role of ActA was demonstrated by expression of *actA* in the closely related species

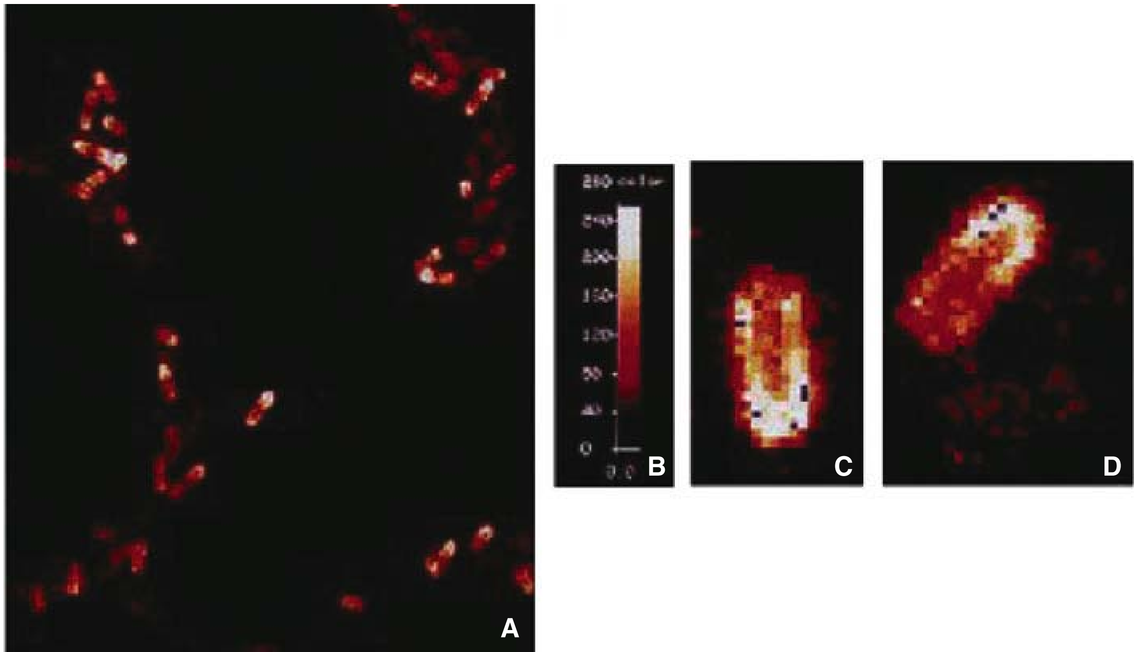

Fig. 35. Polarized distribution of ActA at the *L. monocytogenes* surface. Confocal microscopy images of *L. monocytogenes* labeled with ActA specific antibodies detected with fluorescent secondary antibodies large view (A) and close up (C and D). Linear scale of fluorescence intensities corresponding to images A, C and D (B). Scale bars, 10 µm (A) and 1 µm (C and D). From Kocks et al. (1993), with permission.

Listeria innocua, a bacterium that is totally nonpathogenic and lives in the environment. *Listeria innocua* expressing *actA* were able to move in cell extracts (Kocks et al., 1995). The final demonstration was achieved by the generation of latex beads coated with ActA, which, like *L. innocua* expressing ActA, were able to move inside cell extracts (Cameron et al., 1999).

Genetic analysis has greatly contributed to the analysis of the ActA protein and its mode of action. It was first shown that the N-terminal part of ActA is the critical part for movement. Bacteria expressing a chimera made of the N-terminal part of ActA and the ω fragment of LacZ can move in cell extracts, demonstrating that the first 233 amino acids are sufficient for movement (Lasa et al., 1995). A series of converging analyses, combined with biochemical approaches, has then demonstrated that this region is structurally and functionally similar to WASP/N-WASP (Wiskott-Aldrich syndrome protein) family proteins of eukaryotic cells and binds actin and Arp2/3 (Lasa et al., 1997; Skoble et al., 2000; Boujemaa-Paterski et al., 2001; Skoble et al., 2001; Auerbuch et al., 2003).

WASP/N-WASP proteins, first identified as mutated proteins in genetic disorders such as the Wiskott-Aldrich syndrome, communicate signals downstream from activated small GTPases to the actin cytoskeleton (Machesky and Insall, 1998). They interact with and activate the multiprotein complex Arp2/3, which is normally inactive in resting cells. Upon activation of cells, e.g. after interaction of a growth factor with its receptor, small GTPases can become activated—they switch from a GDP form to a GTP form—and are thus able to interact directly or indirectly with WASP/N-WASP family proteins, which in turn recruit and activate the Arp2/3 complex. This complex is a seven-protein complex able to stimulate de novo actin nucleation and the generation of a branched meshwork of actin filaments that grow from the sides of existing filaments leading to distinctive 70° branches. The C-terminal part of WASP/N-WASP proteins (called VCA or CA domain) stimulates the actin nucleating activity of Arp2/3 complex. This VCA domain is made of a N-terminal verprolin homology region, which binds actin (V or AB for actin binding in Fig. 34), a hydrophobic region termed the central region C, and a C-terminal segment rich in aspartate and glutamate termed the acidic region A in (Fig. 34). The primary Arp2/3 binding site is the A region, while the C region acting in concert with the V region and a bound actin monomer may drive the conformational changes necessary to stimulate nucleation (Machesky and Insall, 1998). Mutations in the 5′ end of the *actA* gene, in the regions encoding peptidic segments similar to the C and A regions, have definitively established similarities between ActA and other Arp2/3 activators. However, it is important to note the amino-acid stretches that bind Arp2/3 and actin in ActA, in contrast to

WASP/N-WASP family proteins, are not contiguous (Pistor et al., 2000; Skoble et al., 2000; Boujemaa-Paterski et al., 2001). Thus *L. monocytogenes* mimics activation of the Arp2/3 complex by WASP/N-WASP family proteins to achieve motility. However, Arp2/3 activation alone is not sufficient to promote actin-based motility in vitro (Loisel et al., 1999). An actin filament capping protein, either CapZ or gelsolin, and the filament depolymerizing and severing protein ADF/cofilin are also required to achieve motility. CapZ or gelsolin ensure that the actin polymerization driving *Listeria* motility is limited to uncapped filaments closely apposed to the bacterium, whereas ADF/cofilin ensures that actin monomers are made available by depolymerizing actin at the pointed end of the filaments in the tail (Loisel et al., 1999) (Fig. 36).

Another important component of the system, although not absolutely essential, is VASP whose absence decreases the rate of motility ten times (Geese et al., 2002). VASP binds to the proline rich region of ActA. VASP by its property to recruit profilin, which in turn facilitates the formation of ATP-actin from ADP-actin, could provide polymerization competent actin monomers to the ActA-Arp2/3 machinery (Grenklo et al., 2003). Other hypotheses have been proposed (Skoble et al., 2001; Bear et al., 2002; Auerbuch et al., 2003; Samarin et al., 2003). VASP by its ability to compete with capping proteins could stimulate filament elongation (Bear et al., 2002). Alternatively, VASP could increase branch spacing (Samarin et al., 2003).

While ActA is now recognized as a bacterial protein mimicking WASP/N-WASP family proteins, it is important to insist on the very instrumental role that this protein has played in deciphering the role of the Arp2/3 complex in actin-based motility. Indeed, while Arp2/3 complex had been isolated from a profilin-sepharose column in 1994 (Machesky et al., 1994), its role had remained totally mysterious until Mitchison and colleagues fractionated platelet cell extracts and showed that the fraction containing the Arp2/3 complex was able to stimulate an actin cloud around ActA-expressing *Listeria* and that ActA was able to activate Arp2/3 (Welch et al., 1997; Welch et al., 1998). The next important step was the two-hybrid approach used by Machesky and colleagues who showed that one of the subunits of the Arp2/3 complex (used as a bait) binds to WASP/N-WASP family proteins (Machesky and Insall, 1998). Then it was shown that WASP coated beads can move in cell extracts as do *Listeria* bacteria (Yarar et al., 1999). There is no better example of such a large contribution of a bacterial protein to the understanding of a key cell biology process, i.e., actin-based motility. How the force generated by the actin polymerization itself is producing movement is now being studied in detail with ActA-coated objects (Bernheim-Groswasser et al., 2002).

Several other bacteria can use actin to move intracellularly. *Listeria ivanovii* uses a protein similar to ActA (Gouin et al., 1995). Interestingly, *Shigella* uses the outer membrane protein IcsA that recruits N-WASP, which in turn recruits and activates Arp2/3 (Egile et al., 1999). In contrast, *Rickettsia* express an outer membrane protein, RickA, that is similar to WAVE, a member of the WASP/N-WASP family proteins and recruits the Arp2/3 complex (Gouin et al., 2004). However, the actin filaments in the *Rickettsia* tails are totally unbranched raising the possibility that another factor is unbranching the filaments or that the RickA protein itself is also unbranching the filaments. Possibly, other bacteria use variations on the theme to promote their intracellular movements. Recent reports

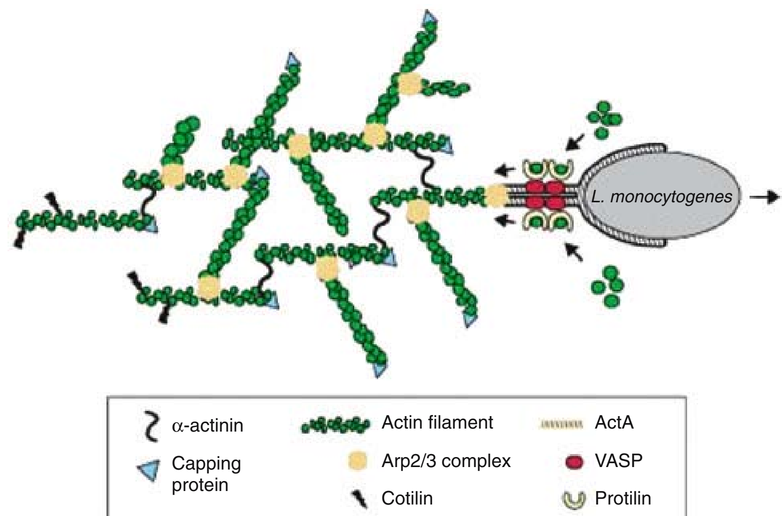

Fig. 36. Model of actin assembly induced by ActA. ActA is represented as a dimer localized in the hatched area in agreement with its polar distribution. The bacterium is moving from left to right (arrow). From Cossart and Bierne (2001), with permission.

indicate that *Mycobacterium marinum* (Stamm et al., 2003) and *Burkholderia pseudomallei* (Breitbach et al., 2003) are other examples of intracellular bacteria able to move inside cells.

Peptidoglycan and Cell Wall Associated Polymers Peptidoglycan (PG) is one of the principal components of the cell wall of Gram-positive bacteria, along with associated polymers, such as the teichoic acids (TAs) and lipoteichoic acids (LTAs) (Fig. 37). It is a thick and rigid structure that covers the plasma membrane, protecting the cytoplasmic content (Merchante et al., 1995). The peptidoglycan and its associated polymers (TAs and LTAs) participate in the maintenance of the bacterial architecture and in anchoring bacterial surface proteins (Jonquieres et al., 1999; Navarre and Schneewind, 1999; Cossart and Jonquieres, 2000). They also possess several biological functions, such as resistance to lysozyme (Kamisango et al., 1982), bacteriophage receptors (Wendlinger et al., 1996), modulator of intracellular signaling in response to infection (Greenberg et al., 1996; Hauf et al., 1997), mitogenicity (Hether et al., 1983b; Paquet et al., 1986), and bacterial adhesion to and invasion of eukaryotic cells (Cowart et al., 1990; Autret et al., 2001).

Composition of the Peptidoglycan The peptidoglycan is a polymer composed of peptides and carbohydrates forming a dense and thick tridimensional network (20–50 nm). The glycans are organized as polymers of *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) residues connected by a β1-4 bond (Fig. 38). The glycan heteropolymers are bound by peptidic bridges through the lactyl group of the Mur*N*Ac residue, allowing the constitution of a reticulate network (Archibald et al., 1993). The peptidic polymers are composed of a pentapeptide L-Ala-D-Glu-*meso*-diaminopimelic acid (*m*DAP)-D-Ala-D-Ala and are connected by a direct transpeptidation of the *m*DAP residue with the D-Ala residue of the peptidic bridges (Schleifer and Kandler, 1972; Fiedler et al., 1988; Archibald et al., 1993).

Synthesis and Degradation of the Peptidoglycan Despite its robustness, the peptidoglycan is not an inert structure and the rapid multiplication of *L. monocytogenes* suggests a rapid turnover for

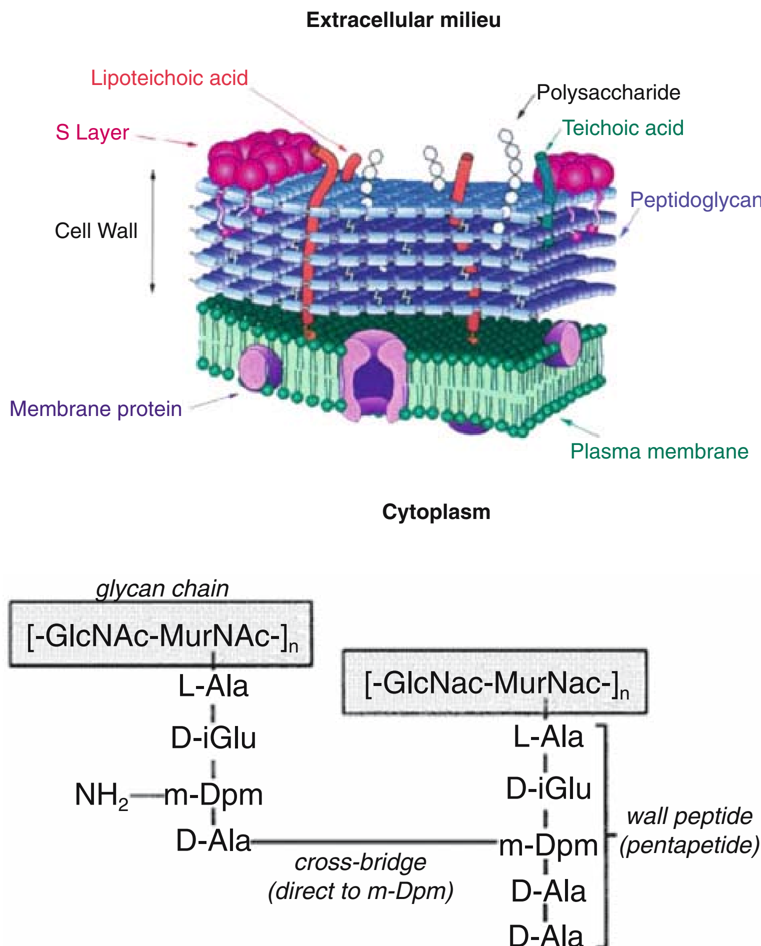

Fig. 37. Model representing a Gram-positive bacterial envelope and its major constituents. Note that a S-layer has not been described in the case of *L. monocytogenes*. From Delcour et al. (1999), with permission.

Fig. 38. Schematic representation of the structure of *L. monocytogenes* peptidoglycan. From Navarre and Schneewind (1999), with permission.

its cell wall components, including the peptidoglycan. The peptidoglycan synthesis occurs as a four-step process. First, there is a sequential ATP-dependent addition of the pentapeptide to the UDP-Mur*N*Ac residues. The newly formed molecule binds the lipid transporter undecaprenol-phosphate to the plasma membrane, which releases the UDP residue. The addition of Glc*N*Ac from the UDP-Glc*N*Ac forms lipid II, which is exported to the external face of the membrane. Finally, the lipid II transfers Glc*N*Ac-Mur*N*Ac-pentapeptide groups to the forming peptidoglycan. The transglycosylation and transpeptidation steps are made by diverse penicillin-binding proteins (Navarre and Schneewind, 1999). The rapid degradation of the peptidoglycan is mediated by autolysins and endolysins, produced by *L. monocytogenes* or its bacteriophages, respectively (Fig. 39). Autolysins and endolysins can cleave different bonds of the peptidoglycan, including the Mur*N*Ac(1-4)Glc*N*Ac (*N*-acetylmuramidases), the Glc*N*Ac(1-4)Mur*N*Ac (*N*-acetylglucosaminidases), the bond between the lactyl group of Mur*N*Ac and the amino group of L -Ala of the branched polypeptide (*N*-acetylmuramoyl-L -alanine amidases), the branched polypeptide at the bond between D-*iso*-Glu and the *m*DAP residues (endopeptidases), and the peptide bridges cross-linking the peptidoglycan between the L-Ala and D-Gln residues (L-alanoyl-D-glutamate peptidases) (Ghuysen et al., 1966; Schleifer and Kandler, 1972).

Composition of the Cell Wall Associated Polymers In *L. monocytogenes*, these polymers comprise TAs and LTAs. In other Gram-positive bacteria, they also include the teichuronic acids and the polysaccharides. These associated polymers are most of the time essential for bacterial growth and viability (Neuhaus and Baddiley, 2003).

TAs are electronegative polymers of ribitol-phosphates covalently bound to the peptidoglycan (Fig. 40). They are substituted by D -Ala residues and diverse sugars, which vary depending on serotypes (Ruhland and Fiedler, 1987; Fiedler et al., 1988; Fischer et al., 1988). LTAs are polymers of glycerophosphate substituted by Ala, galactose, and lipid residues (Fig. 41).

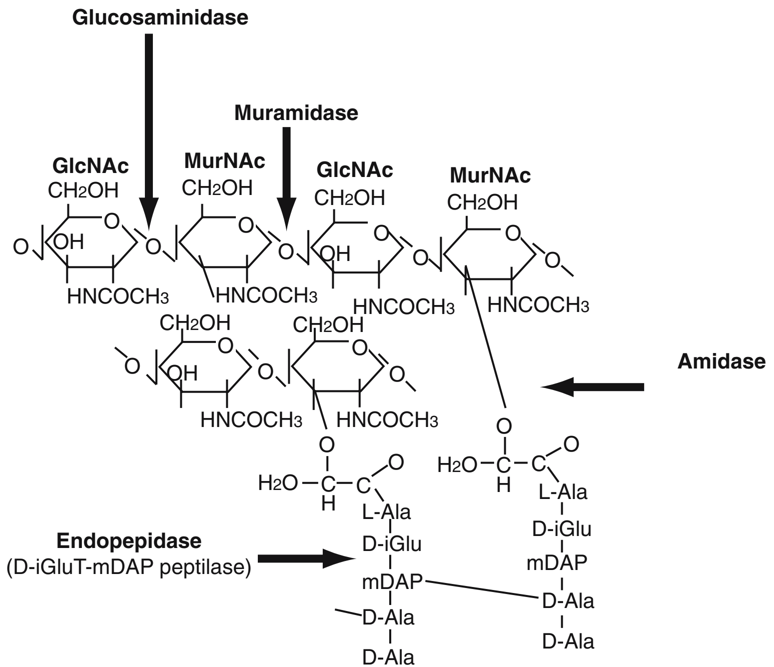

Fig. 39. Schematic representation of autolysin cleavage sites in *L. monocytogenes* peptidoglycan. From Lenz et al. (2003), with permission.

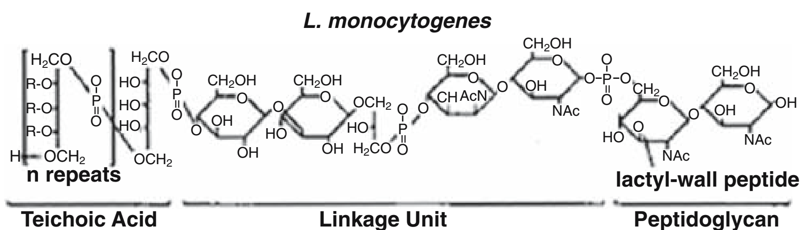

Fig. 40. Schematic representation of the structure of *L. monocytogenes* teichoic acids as well as the linking unit connecting them to the peptidoglycan. From Navarre and Schneewind (1999), with permission.

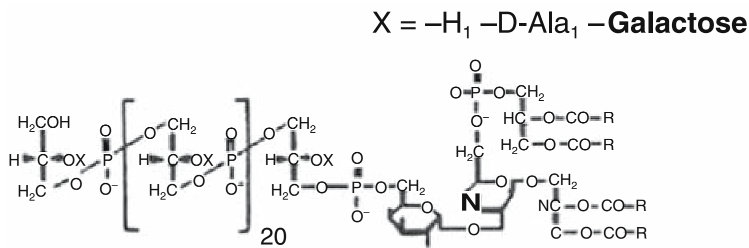

Fig. 41. Schematic representation of the structure of *L. monocytogenes* lipoteichoic acids. From Greenberg et al. (1996), with permission.

Recently, the *dlt* operon, which catalyzes the incorporation of D -Ala residues into LTAs, has been characterized. It comprises four genes, *dltA* to *dltD*, with *dltA* supposedly encoding the D-alanine-D-alanyl carrier protein ligase (Abachin et al., 2002). The glycerophosphate chain of LTAs is uncovalently anchored to the outer leaflet of the plasma membrane through a lipid anchor, composed of galactose bound to glycerol and substituted by fatty acids (Gal[α1-2]Glc[α 1-3]diacyl-glycerol) (Hether and Jackson, 1983a; Ruhland and Fiedler, 1987; Greenberg et al., 1996). Contrary to TA, the LTA structure does not vary with serotypes (Fiedler et al., 1988). However, in some *Listeria* strains, LTAs possess an additional phosphatidyl residue, which strengthens their association with the plasma membrane (Fischer et al., 1990). The LTAs are both membrane associated and secreted in the growth medium (Fischer et al., 1988). Their X-ray structure suggests that they adopt a compact organized micellar form with a length of 10–20 nm. Since the cell wall thickness ranges from 20 to 50 nm, some membrane LTAs are probably not exposed to the external medium (Labischinski et al., 1991).

Functions and Biological Activities of the Peptidoglycan and Associated Polymers

Functions Important for Bacterial Structure Probably the most important function of PG and its associated polymers, TAs and LTAs, resides in their participation in the maintenance of the cell wall and bacterial structures. Because of their negative charges, TAs are proposed to participate in the maintenance of a polarized membrane, as well as an electrochemical gradient within the cell wall, which locally concentrates certain ions (Hugues et al., 1973; Jolliffe et al., 1981; Kemper et al., 1993). Similarly, LTAs appear to favor the local concentration of Mg^{2+} ions, which may stabilize the plasma membrane (Roberts et al., 1985). LTAs regulate the activity of autolysins, which are involved in the peptidoglycan degradation required for bacterial growth and division (Fischer et al., 1981).

Role in Anchoring Bacterial Surface Proteins and as Bacteriophage Receptors PG and LTAs are also essential in that they anchor several *L. monocytogenes* surface proteins. Indeed, LPTXG-containing proteins, such as certain internalins, are covalently anchored to the PG allowing their exposure at the bacterial surface. This anchoring is mediated by SrtA (Navarre and Schneewind, 1999). Recently, another sortase, sortase B (SrtB), has been characterized in *L. monocytogenes*. It is required for the anchoring of a small subset of surface anchored proteins and probably recognizes its targets through a NXZTN sorting motif (Bierne et al., 2004). The gene encoding SrtB (*srtB*) is homologous to the *Staphylococcus aureus srtB* gene (Mazmanian et al., 2002). Both are part of an operon that contains at least one of their respective targets and exhibits a Fur (ferric uptake regulator) box, suggesting that their expression might be regulated by iron. One of the substrates of SrtB is the surface protein SvpA (Bierne et al., 2004), which was proposed to act as a virulence factor of *L. monocytogenes* (Borezee et al., 2001). By analogy with the SrtB of *S. aureus*, *L. monocytogenes* SrtB was proposed to be involved in bacterial adaptation to environmental conditions (Mazmanian et al., 2002; Bierne et al., 2004). InlB binds to the LTAs through its GW modules, although this noncovalent linkage appears to be relatively labile since InlB can also be released in the medium (Braun et al., 1998; Jonquieres et al., 1999). Ami, an autolysin involved in bacterial adhesion, also binds to the bacterial surface through its GW modules (Braun et al., 1997). It has also been suggested that the ribose groups of TAs and their associated cations may stabilize external bacterial proteins, protecting them from proteolysis (Chambert and Petit-Glatron, 1999; Hyyrylainen et al., 2000). Finally, the glycan residues of PG and TAs act as receptors for bacteriophages at the surface of *L. monocytogenes* (Wendlinger et al., 1996).

Role in Infection and Immunity In addition to its role in resistance to lysozyme (Kamisango et al., 1982), many reports point to a role of PG in stimulating the immune response. PG was shown early on to activate macrophages and to have

some mitogenic and adjuvant activities (Saiki et al., 1982; Hether et al., 1983b; Paquet et al., 1986). PG induces the production of the migration inhibition factor (Paquet et al., 1986). It enhances the NK activity in vivo and the generation of cell-mediated toxicity against tumor target cells (Saiki et al., 1982; Paquet et al., 1986). PGs were initially proposed to interact with the Toll-like receptor TLR2. However, the use of highly purified fraction of *L. monocytogenes* PGs revealed that they are detected intracellularly by the Nod2 protein and that LTAs contaminants contained in PGs preparations were sensed by TLR2 (Chamaillard et al., 2003; Girardin et al., 2003; Travassos et al., 2004).

TAs share some of the PG properties. They activate macrophages and are mitogenic (Hether et al., 1983b; Paquet et al., 1986). Interestingly, *L. monocytogenes* TAs appear to play a role in adhesion to and entry into epithelial cells. It was recently shown that the glycosylation of TAs favor these processes (Cowart et al., 1990; Autret et al., 2001).

LTAs bind to scavenger receptors on macrophages (Greenberg et al., 1996) and are sensed by TLR2 on epithelial cells (Travassos et al., 2004). In the *L. monocytogenes* strain LO28, a *dltA* mutant affected in the ability to incorporate D-Ala residues into LTAs is less virulent in the mouse model and less adherent to macrophages and epithelial cells (Abachin et al., 2002), suggesting a major role for peptidoglycan in virulence. However, deletion of the *dltA* gene in strain EGD seems to have less effect (Mandin et al., 2005).

AUTOLYSINS AND ENDOLYSINS *Listeria monocytogenes* produces several autolysins that digest its own cell wall peptidoglycan and are therefore listeriolytic. *Listeria monocytogenes* autolysins are proposed to be involved in numerous cellular processes including cell growth and division, septation, cell wall turnover, and peptidoglycan maturation, motility, protein secretion, and virulence (Ward and Williamson, 1984; Blackman et al., 1998; Smith et al., 2000). In addition, certain bacteriophages of *L. monocytogenes* produce endolysins, which are cell wall hydrolases synthesized during late gene expression of the lytic cycle. Endolysins enable the release of progeny virions from infected bacteria through degradation of the bacterial peptidoglycan (Loessner and Scherer, 1995a).

The profile of *L. monocytogenes* peptidoglycan hydrolases comprises multiple bacteriolytic enzymes, as determined by using renaturing SDS gel electrophoresis, with gels containing different bacterial cell wall extracts. Interestingly, the bacteriolytic profiles of different *Listeria* species appear very heterogeneous, highlighting the specificity of these enzymes (MacLaughlan and Foster, 1997). Several *L. monocytogenes* autolysins have been more characterized, including p60, p45, Ami, NamA/MurA, and Auto (Wuenscher et al., 1993; Schubert et al., 2000; Carroll et al., 2003; Lenz et al., 2003; Cabanes et al., 2004). In addition, the *L. monocytogenes* genome sequence determination revealed seven other putative autolysins, with amidase or glucosamidase homology domains (Glaser et al., 2001; Cabanes et al., 2004) (Fig. 42).

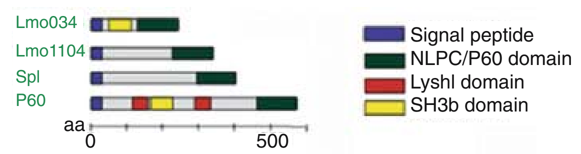

Fig. 42. Alignment of p60-like proteins. Determined from the annotation of the *L. monocytogenes* genome sequence. From Cabanes et al. (2002), with permission.

Autolysin and Endolysin Activities The peptidoglycan of *L. monocytogenes* is composed of a copolymer of Glc*N*Ac alternating with Mur*N*Ac, from which are branched polypeptide side chains. Crosslinks result from transpeptidation between the D -alanine of one peptide side chain and the free amino group of the *m*DAP of another (Dhar et al., 2000). Autolysins and endolysins are classified according to their hydrolytic specificity towards the peptidoglycan bonds (Fig. 39). *N*-acetylmuramidases and *N*-acetylglucosaminidases cleave the Mur*N*Ac(1-4)Glc*N*Ac or the Glc*N*Ac(1-4)Mur*N*Ac bonds, respectively. *N*-acetylmuramoyl-L-alanine amidases cleave the bond between the lactyl group of Mur*N*Ac and the amino group of L-Ala of the branched polypeptide. Endopeptidases directly cleave the branched polypeptide at the bond between D-*i*Glu (isoglutamine) and the *m*DAP residues (Ghuysen et al., 1966). L-Alanoyl-D-glutamate peptidases cleave the peptide bridges crosslinking the peptidoglycan between the L-Ala and D-Gln residues (Schleifer and Kandler, 1972).

Autolysins

p60 The autolysin p60 is encoded by the *iap* (invasion-associated protein) gene, which is transcribed independently of the transcriptional activator PrfA and whose expression is controlled at the post-transcriptional level (Kohler et al., 1991; Bubert et al., 1997; Bubert et al., 1999). It is both secreted and associated with the bacterial cell

wall (Kuhn and Goebel, 1989; Ruhland et al., 1993; Wuenscher et al., 1993). Secretion is mediated by the recently identified auxiliary secretion system SecA2, which mediates the secretion of at least seventeen secreted and surface proteins of *L. monocytogenes* (Lenz et al., 2003). p60 is expressed by all *Listeria* species with specific protein sequences for each species (Bubert et al., 1992b; Bubert et al., 1994; Gutekunst et al., 1992a). This finding can be exploited as a way to identify *Listeria* species (Bubert et al., 1992a; Bubert et al., 1994). On the basis of its similarity to LytF from *Bacillus subtilis*, p60 is proposed to have a D-*i*Glu-*m*DAP endopeptidase activity (Lenz et al., 2003).

In agreement with its peptidoglycan activity, p60 has been shown to be important for septum formation during bacterial division and therefore for bacterial viability (Wuenscher et al., 1993). For a long time, Δ*iap* mutants could not been obtained, suggesting that the protein was essential for bacterial viability. Therefore, the role of p60 was first evaluated in rough mutants expressing lower levels of p60 and forming long filamentous structures composed of bacterial chains (Kuhn and Goebel, 1989). The rough mutants are less virulent and enter less efficiently in certain eukaryotic cells, suggesting a role for p60 in bacterial invasion (Kuhn and Goebel, 1989; Gutekunst et al., 1992b; Hess et al., 1995). A viable Δ*iap* mutant was recently obtained, allowing more precise studies of the role of p60 in virulence. As for rough mutants, they also had a defect in septum formation and in virulence after intravenous infection of mice. In addition, the Δ*iap* mutant is impaired in bacterial movement and spreading from cell to cell because of an improper localization of the ActA at the surface *L. monocytogenes*. This abolishes the ability of ActA to polarly polymerize actin and to generate normal comet tails (Lenz et al., 2003; Pilgrim et al., 2003a).

p60 plays an important role in the immune response towards *L. monocytogenes* infection. Antibodies specific for p60 can act as opsonins and may play a role in preventing systemic infections in immunocompetent individuals (Kolb-Maurer et al., 2001). Moreover, p60 is a major protective antigen that induces both T-CD8 and Th1 protective immune responses, highlighting that both cellular and humoral immunity are important to fight *L. monocytogenes* infection (Harty and Pamer, 1995; Bouwer and Hinrichs, 1996; Geginat et al., 1998; Geginat et al., 1999).

p45 The autolysin p45, encoded by the *spl* gene, was identified using a monoclonal antibody raised in mice against heat-killed bacteria. It is a 45-kDa protein with peptidoglycan lytic activity against *L. monocytogenes*. p45 is homologous to the autolysin p60 of *L. monocytogenes* and to Gram-positive secreted proteins of unknown activity, P54 of *Enterococcus faecium*, and Usp45 of *Lactococcus lactis*. p45-like proteins are present in all *Listeria* species, except *L. grayi*. p45 is found both as a secreted protein, as expected from the presence of a 27-amino acid signal peptide, and associated with the cell wall, in a strong but noncovalent fashion, as determined by SDS extraction. This behavior is reminiscent of p60 and of the two main effectors of *L. monocytogenes* invasion, InlA and InlB (Schubert et al., 2000).

Ami The autolysin Ami is a 102-kDa protein produced by *L. monocytogenes*. It is probably an amidase according to the sequence homology of its catalytic domain with other amidase domains (Braun et al., 1997). It is associated to the bacterial surface by a domain containing a series of GW modules, similar to those of one of the major proteins required for *L. monocytogenes* invasion, InlB (Braun et al., 1997; Jonquieres et al., 1999) (Fig. 19B). There is increasing evidence that certain autolysins, like Ami, may act as complementary adhesins during infection. Indeed, inactivation of Ami in mutants devoid of either one or two of the main effectors of *L. monocytogenes* invasion, InlA and/or InlB, resulted in a strong reduction of adhesion to hepatocytes, HepG2, and to the enterocyte-like cell line, Caco-2. On the contrary, inactivation of Ami alone affected only slightly the adhesion to hepatocytes, HepG2 (Milohanic et al., 2001). As for InlB, the cell wall-anchoring (CWA) domain containing the GW modules appears to promote Ami adhesion to cells (Heilmann et al., 1997; Hell et al., 1998; Milohanic et al., 2001). As a probable consequence of its role in adhesion, a mutant with inactivated Ami is slightly attenuated in the liver of mice infected intravenously, indicating a role for Ami in *L. monocytogenes* virulence (Milohanic et al., 2001). Ami from two different epidemic serovars (1/2a and 4b) were compared and their CWA domains were shown to be variable, as compared with the rest of the molecule. The purified CWA domain of Ami from serovar 4b binds less efficiently to Hep-G2 cells as compared with that of serovar 1/2a (Milohanic et al., 2004).

NamA/MurA Two recent concomitant studies identified a new peptidoglycan-hydrolyzing enzyme in *L. monocytogenes*, which was named by the authors "NamA" for *N*-acetylmuramidase or "MurA" for muramidase (Carroll et al., 2003; Lenz et al., 2003). Comparative analysis of biochemical and genetic properties of the two molecules revealed NamA is the same protein as MurA (D. Cabanes, personal communication).

Characterization of the auxiliary protein secretion system, SecA2, revealed that the secretion of several proteins was dependent on this system, including the autolysin p60 and a protein homologous to NamA (Lenz et al., 2003). In parallel, a cell wall hydrolase (MurA) encoded by the *murA* gene was detected by a *L. monocytogenes*-specific monoclonal antibody (EM-7G1), which also recognizes the autolysin p60 (Carroll et al., 2003).

MurA is a major cell surface protein of *L. monocytogenes*. The C-terminal domain of MurA contains four copies of a KM repeat motif, which is also present in p60 and is proposed to be involved in cell wall anchoring. MurA shares homologies with the autolysin p60 and with muramidases. In agreement with this, MurA exhibits a peptidoglycan lytic activity specific for *Micrococcus lysodeikticus*. A deletion mutant of *mur*A lacking the cell wall hydrolase activity is affected in *L. monocytogenes* autolysis and grows as long chains during exponential growth, as a consequence of a septation defect (Carroll et al., 2003). The role of MurA in cell infection and in virulence was not tested in this study, but the study of NamA revealed a moderate role in *L. monocytogenes* persistence in mouse organs (Lenz et al., 2003).

Auto Recently, a novel autolysin encoding gene, *aut*, was identified. It is the only autolysin gene that is absent from the nonpathogenic bacteria *L. innocua.* The *aut* gene is expressed independently of the virulence gene regulator PrfA and encodes a surface protein, Auto, with an autolytic activity, as expected from the presence of a domain harboring homologies with autolysin encoding genes, especially *N*-acetylglucosaminidases. The protein Auto possesses a C-terminal cell wall-anchoring domain made up of four GW modules, similar to those observed in the other autolysin Ami and in InlB, one of the major invasion proteins of *L. monocytogenes*. The morphology of a Δ*aut* deletion mutant was similar to those of the wild-type, with no defect in septation and cell division, suggesting no role for Auto in these functions (Cabanes et al., 2004).

Auto is required for entry of *L. monocytogenes* into different nonphagocytic eukaryotic cell lines but is not required for efficient adhesion, formation of comet tails, or cell-to-cell spreading. A Δ*aut* deletion mutant has a reduced virulence following intravenous inoculation of mice and oral infection of guinea pigs, which correlates with its low invasiveness. However, the autolytic activity of Auto by itself, rather than an invasive ability, might be critical for virulence. Indeed, Auto may control the general surface architecture exposed to the host by *L. monocytogenes* and/or the composition of the surface products released by the bacteria, highlighting the possible direct role of autolysins in pathogenicity (Cabanes et al., 2004).

FlaA *Listeria monocytogenes* can move by means of flagella-based motility. The flagellum is composed of a single protein, FlaA (Dons et al., 1992). FlaA also facilitates initial contact with epithelial cells and contributes to effective invasion in vitro (Dons et al., 2004). It was shown that the purified FlaA protein functions as a peptidoglycan hydrolase (Popowska and Markiewicz, 2004). The flagellar protein FlgJ of *Salmonella*, was previously shown to have peptidoglycan hydrolyzing activity, locally digesting the murein sacculus to permit assembly of the rod structure of the flagellum (Nambu et al., 1999). FlaA of *L. monocytogenes* is the first gram-positive flagellar protein demonstrated to have a peptidoglycan hydrolyzing activity.

Endolysins Three endolysins, Ply118, Ply500 and Ply511, produced respectively by the bacteriophages A118, A500 and A511, have been characterized in *L. monocytogenes*. They induce rapid lysis of *Listeria* strains from all species but not of other bacteria (Loessner and Scherer, 1995a). Ply511 is an *N*-acetylmuramoyl-L-alanine amidase, while Ply118 and Ply500 are L-alanoyl-D-glutamate peptidases (Schleifer and Kandler, 1972). A cell wall binding site has been characterized in the C-terminal domains of Ply118 and Ply500 as being sufficient to direct the enzymes to their substrates (Loessner et al., 2002). *Listeria innocua* carries a cryptic phage 2438, which produces the Cpl2438 enzyme highly similar to Ply500 (Zink et al., 1995). A gene homologous to the Ply118 endolysin was detected in the genome sequence of the cryptic phage of *L. monocytogenes* strain EGDe (Glaser et al., 2001). The specificity of these endolysins for *Listeria* cells was exploited in different applications, such as rapid in vitro lysis of *Listeria* cells (Loessner et al., 1995b; Dhar et al., 2000), or programmed self-destruction of intracellular *Listeria* cells within the cytosol of macrophages (Dietrich et al., 1998).

OTHER FACTORS INVOLVED IN *L. MONOCYTOGENES* INFECTION Several factors have been involved in the *L. monocytogenes* infection process either at the level of adhesion, entry, escape from the phagosome, or intracellular multiplication, or at an unknown step.

LAP LAP (*Listeria* adhesion protein) is a 104-kDa protein present both in the cytoplasm and secreted by *L. monocytogenes*. LAP is expressed by all *Listeria* spp. except by *L. grayi* (Pandiripally et al., 1999). LAP mediates *L.*

monocytogenes binding to intestinal epithelial cells but not to nonintestinal epithelial cells, such as liver, kidney or skin cells. Interestingly, the specificity of LAP is even more restricted since it is required for full adhesion to intestinal epithelial cells lines from the ileum-cecum and colon but not from the duodenum or jejunum. These results suggest that LAP may play a role during the intestinal phase of the infection (Jaradat et al., 2003). The heat shock protein Hsp60 has been reported to act as a cellular receptor for LAP on the intestinal cell line Caco-2 (Wampler et al., 2004).

SvpA SvpA (surface virulence-associated protein) is a 64-kDa protein both anchored at the bacterial surface by SrtB and secreted in the extracellular medium (Borezee et al., 2001; Bierne et al., 2004). Its expression is not controlled by PrfA, the transcriptional activator of most virulence factors. A mutant deficient in SvpA was reported to be less virulent in the mouse model after intravenous infection (Borezee et al., 2001). However, the relevance of this result is unclear since virulence of a mutant deficient in SrtB, in which SvpA is no longer exposed at the surface, is not affected (Bierne et al., 2004). The growth defect of the *svpA* mutant may explain its virulence attenuation (H. Bierne and P. Cossart, unpublished results). Another possibility is that the expression of SvpA at the bacterial surface is not important for its role in virulence.

LpeA LpeA (lipoprotein promoting entry) is a 35-kDa protein identified by in silico analysis of the *L. monocytogenes* genome sequence. Despite its homology with PsaA, a *Streptococcus pneumoniae* adhesin, LpeA is not involved in adhesion of *L. monocytogenes*. Instead, it is required for entry into the intestinal and hepatic cell lines. Interestingly, a LpeA-deficient mutant survive longer in macrophages than wild-type bacteria and is slightly more virulent for mice (Reglier-Poupet et al., 2003b).

FbpA FbpA (fibronectin binding protein) is a 60-kDa protein identified by signature-tagged mutagenesis. It is required for efficient liver colonization of mice inoculated intravenously and for intestinal and liver colonization after oral infection of transgenic mice expressing human E-cadherin. FbpA binds to immobilized human fibronectin and increases adherence of wild-type *L. monocytogenes* to HEp-2 cells in the presence of exogenous fibronectin. Despite the lack of conventional secretion/anchoring signals, FbpA is detected on the bacterial surface. Strikingly, FbpA expression affects the secretion of two virulence factors, LLO and InlB, and coimmunoprecipitates with these two proteins. Thus, FbpA, in addition to being a fibronectin-binding protein, may behave as a chaperone or an escort protein for two important virulence factors and appears to be a novel multifunctional virulence factor of *L. monocytogenes* (Dramsi et al., 2004).

Stp Analysis of the *L. monocytogenes* EGDe genome sequence revealed the presence of putative eukaryotic-like phosphatases. Accordingly, the *stp* gene encodes a membrane associated Mn^{2+}-dependent serine-threonine phosphatase, required for *L. monocytogenes* virulence. Using a phosphoproteomic approach, the translation elongation factor EF-Tu was identified as the first target of Stp (Archambaud et al., 2004).

Vip Vip is a novel virulence factor of *L. monocytogenes*, identified by comparative genomics. Vip is a LPXTG surface protein anchored to the cell wall by sortase A. It is positively regulated by PrfA, the transcriptional activator of the major *L. monocytogenes* virulence factors. Vip is implicated in entry into some mammalian cells. Gp96, an endoplasmic reticulum resident chaperone protein (Li et al., 2002), which is also localized at the cell surface, was identified as a cellular receptor for Vip (Cabanes et al., 2005). The Vip-Gp96 interaction is critical for bacterial entry into cells. In murine models, Vip plays a role in *L. monocytogenes* virulence at the intestinal level and at late stages of the infectious process. Vip appears as a new virulence factor exploiting Gp96 as a receptor for cell invasion and/or signaling events that may interfere with the host immune response in the course of *Listeria* infection (Cabanes et al., 2005).

Resistance to Stress *Listeria monocytogenes* is ubiquitous in nature and can infect many animal species. The pathogenic bacterium is adapted to survive and/or multiply under a wide variety of harsh environmental conditions, outside as well as inside the host. In food-processing plants, food products, and nature, *L. monocytogenes* has to contend with suboptimal growth conditions, such as refrigeration temperatures, heat, high osmolarity, high pH, and low water activity. Survival has been reported from –0.4°C to 45°C (Farber and Peterkin, 1991a), at salt concentrations up to 10% (McClure et al., 1991), alkaline conditions up to pH 9 (Cheroutre-Vialette et al., 1998). In the human host, *L. monocytogenes* encounters stressors such as lysozyme in saliva, blood, neutrophils and monocytes, reactive oxygen and nitrogen species in the host cell phagosomes, bile salts, digestive enzymes in the small intestine, and stressful microenvironments (such as nutrient deprivation in the host cell cytoplasm, acidic

pH of the stomach, and low oxygen tension and high osmolarity in the intestine). *Listeria monocytogenes* survives and/or grows at a pH as low as 4, even 3 after acid adaptation (Cotter and Hill, 2003) and in the presence of a high concentration of bile (Begley et al., 2002). *Listeria monocytogenes* has evolved a series of adaptive responses to cope with this large variety of stresses.

The first step in stress survival strategies is the sensing of stress conditions and/or stressors and the transduction of a signal leading to an appropriate response (Fig. 43). Two-component signal transduction systems are used for stress sensing by many bacteria, including *L. monocytogenes*. Deletion of *L. monocytogenes* LO28 *lisK*, the gene encoding the membrane-associated sensor histidine kinase of the LisRK two-component system, affects acid and ethanol tolerance and attenuates virulence in mice (Cotter et al., 1999). Using a deletion mutant of the response regulator gene *lisR*, Kallipolitis et al. confirmed the role of the LisRK system in virulence, acid and ethanol tolerance. LisR was also shown to be involved in response to oxidative stress, as growth of the *lisR* mutant was strongly inhibited by 0.025% hydrogen peroxide. Two other response regulators contribute to pathogenicity and stress response in *L. monocytogenes* LO28 strain, a regulator showing 52% identity with the potassium uptake protein KdpE of *Clostridium acetobutylicum* and a regulator showing 43% identity with the *Enterococcus faecium* VanR protein involved in vancomycin resistance (Kallipolitis and Ingmer, 2001).

Many stress response mediators have been identified in *L. monocytogenes*, including chaperones that maintain protein integrity under damaging conditions and proteases that degrade damaged proteins. The DnaK heat shock chaperone is required for survival of *L. monocytogenes* under high temperatures and acidic conditions, as well as for phagocytosis by macrophages (Hanawa et al., 1995; Hanawa et al., 1999). The major heat shock chaperones GroES and GroEL are induced at high temperature (Hanawa et al., 1995; Gahan et al., 2001), at low pH and during infection of J774 cells (Gahan et al., 2001). The ClpC stress protein, an ATPase that belongs to the HSP-100/Clp family, is required for survival under iron deprivation, high temperature or high osmolarity, in bone marrow macrophages and in organs of mice infected by *L. monocytogenes* (Rouquette et al., 1996; Rouquette et al., 1998). Two other proteases have been reported to be involved in stress response and virulence, namely the HSP-100 family member ClpE (Nair et al., 1999) and the serine protease ClpP (Gaillot et al., 2000). Several other proteins involved in specific stress responses have been characterized in *L. monocytogenes*. The major cold shock protein of *L. monocytogenes* is the nonheme ferritin-like protein Flp, also designated Fri (Hebraud and Guzzo, 2000). Fri promotes adaptation to nutritional and thermal shifts, provides protection against reactive oxygen species *in vitro* and is required for full virulence of *L. monocytogenes* (Olsen et al., 2005; Dussurget et al., 2005). The glutamate decarboxylase system plays an important role in survival at low pH found in food (Cotter et al., 2001b) and in gastric fluid (Cotter et al., 2001a). The F_0F_1-ATPase could also contribute to acid tolerance of *L. monocytogenes*

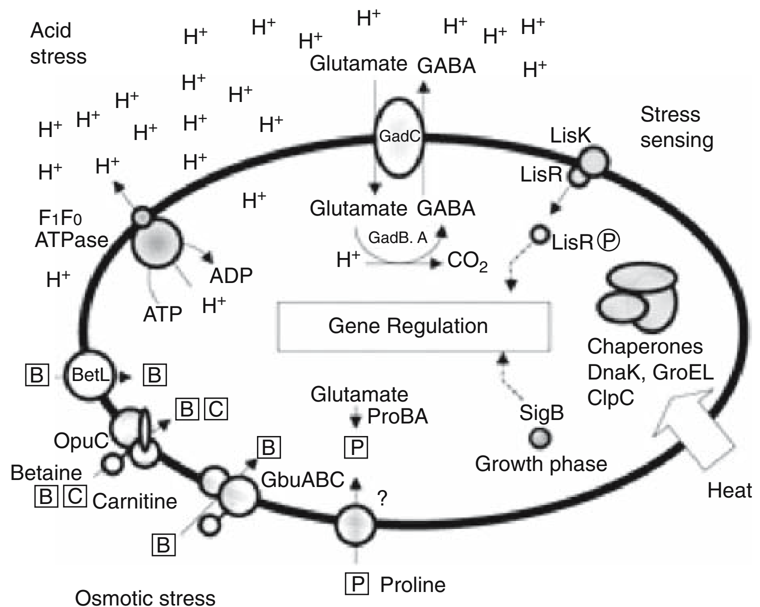

Fig. 43. Schematic representation of the stress resistance process in *L. monocytogenes*. From Hill et al. (2002), with permission.

(Cotter et al., 2000). Adaptation to osmotic stress depends on the intracellular accumulation of osmolytes, e.g., betaine, carnitine and proline (Ko et al., 1994; Fraser et al., 2000). Uptake systems include the Na^+-dependent betaine porter I BetL (Sleator et al., 1999), the ATP-dependent betaine porter II GbuABC (Ko and Smith, 1999), the carnitine uptake systems OpuC (Fraser et al., 2000; Sleator et al., 2001a; Sleator et al., 2003; Wemekamp-Kamphuis et al., 2002), and the oligopeptide permease OppA (Borezee et al., 2000). In addition, one osmolyte synthesis system has been described in *L. monocytogenes*, that of proline. Mutation of the *proAB* operon leads to an increased sensitivity to salt (Sleator et al., 2001a). Several of these systems have been shown to play a role in the virulence potential of *L. monocytogenes*, e.g., OpuC (Sleator et al., 2001b) and OppA (Borezee et al., 2000). Bile tolerance of *L. monocytogenes* involves the bile salt hydrolase Bsh, an enzyme that deconjugates bile salts and that is required for both intestinal and hepatic phases of listeriosis (Dussurget et al., 2002; Begley et al., 2005). The transporter BtlA (Begley et al., 2003) and other systems (such as the putative transporter of the glutamate decarboxylase GadE, the penicillin V amidase Pva, the bile tolerance protein BtlB, the bile exclusion system BilE and the zinc uptake regulator ZurR) contribute to tolerance to bile or various other stresses, e.g., low pH, salt, ethanol, detergents and antibiotics (Begley et al., 2002; Sleator et al., 2005a; Begley et al., 2005).

Stress-responsive sigma factors play an important role in regulating expression of both virulence genes and stress response genes in bacteria, including *L. monocytogenes*. The alternative sigma factor sigma B contributes to the ability of *L. monocytogenes* to survive and/or multiply under stressful conditions outside the host, e.g., acid, osmotic or oxidative stresses (Becker et al., 1998; Ferreira et al., 2001; Ferreira et al., 2003), low temperature (Becker et al., 2000) or carbon starvation (Ferreira et al., 2001). Sigma B also plays a role in the capacity of *L. monocytogenes* to persist within the host during the infectious process. It has been demonstrated that sigma B contributes to transcription of the virulence gene activator PrfA (Nadon et al., 2002). Characterization of the sigma B-dependent general stress regulon confirmed the broad role of this sigma factor. Indeed, the regulon includes genes encoding both general stress response proteins (e.g., the RNA-binding protein Hfq, glutamate decarboxylase GadB, general stress protein Ctc, and the glutathione reductase), and virulence factors (e.g., the internalins InlA and InlB, the bile salt hydrolase Bsh, the bile exclusion system BilA and the stress-responsive solute transporter OpuC (Sleator et al., 2001b; Kazmierczak et al., 2003; Sue et al., 2003; Christiansen et al., 2004; Kim et al., 2004; Sleator et al., 2005a). *Listeria monocytogenes* stress response is also controlled by CtsR, a transcriptional repressor of *clpC*, *clpE* and *clpP*. A *ctsR* deletion mutant was not affected for virulence in mice and displayed an increased survival at high temperature and under salt stress (Nair et al., 2000). Finally, the regulatory RNA-binding protein Hfq is required for resistance to osmotic and ethanol stress, and full virulence in mice (Christiansen et al., 2004).

Gene Regulation in *Listeria*

Analysis of the complete genome sequence of *L. monocytogenes* EGDe revealed its extraordinary regulatory capacity as 201 regulatory proteins have been identified (Glaser et al., 2001). The *L. monocytogenes* genome contains the highest proportion of regulatory genes (7%) after that of *Pseudomonas aeruginosa* (8.4%) (Stover et al., 2000). This observation is in line with the fact that *L. monocytogenes* is an ubiquitous, opportunistic pathogen that needs a variety of combinatorial pathways to adapt its metabolism to a given niche. However, only a few of these regulators have been studied experimentally.

Two-Component Systems The *L. monocytogenes* genome contains several two-component systems consisting of 15 histidine kinases and 16 response regulators. Several of them have been studied in detail: *lisR*/*lisK* (Cotter et al., 1999; Cotter et al., 2002), *cheY*/*cheA* (Flanary et al., 1999), *agrA*/*agrC* (Autret et al., 2003), *cesR*/*cesK* (Kallipolitis et al., 2003) and *virS/virR* (Mandin et al., 2005). The LisR/LisK system is involved in acid, ethanol and oxidative stress, and the inactivation of *lisRK* resulted in a slight decrease of virulence (Cotter et al., 1999). The LisRK signal transduction system is also involved in the sensitivity of *L. monocytogenes* to nisin and cephalosporins (Cotter et al., 2002). The genes regulated by this system are a putative penicillin-binding protein (*lmo2229*), a histidine kinase (*lmo1021*), and a protein of unknown function (*lmo2487*). A novel role for the two-component regulatory system LisRK in osmosensing and osmoregulation has been shown (Sleator and Hill, 2005b). Furthermore, *htrA*, a gene linked to osmotolerance and virulence potential in *L. monocytogenes*, was reported to be under the transcriptional control of LisRK (Stack et al., 2005).

Insertional inactivation of the *L. monocytogenes cheYA* operon abolished response to oxygen gradients and reduced flagellin expression and affected the ability of *L. monocytogenes* to attach to the mouse fibroblast cell line 3T3 (Flanary et al., 1999). A deletion mutant *cheA*

had impaired swarming and the *cheY* and *cheYA* double mutants were unable to swarm on soft agar plates, suggesting that *cheY* and *chaA* genes encode proteins involved in chemotaxis (Dons et al., 2004). Autret and colleagues identified by signature-tagged mutagenesis the *agrABCD* locus (Autret et al., 2003). The production of several secreted proteins was modified, indicating that the *agr* locus influenced protein secretion. Although the ability of the mutant to invade and multiply in cells in vitro was not affected, virulence of the *agrA* mutant was affected in the mouse model, indicating that the *agr* locus is involved in virulence of *L. monocytogenes* (Autret et al., 2003).

Kallipolitis and colleagues inactivated five putative response regulators identified by using degenerate primers in *L. monocytogenes* strain LO28 (Kallipolitis et al., 2003). These correspond to the genes *lmo2583*, *lmo2678/kdpE*, *lmo2501/phoP*, *lmo2422/cesR*, and *lmo1377/lisR*. Three of these putative response regulators, *lmo2678/kdpE*, *lmo2422/cesR*, and *lmo1377/lisR*, contributed to pathogenicity in a mouse infection model either by intragastric or intraperitoneal injection. Recently, *cesRK* was studied in more detail (Kallipolitis et al., 2003), showing that this two-component system responds to the presence of cell wall-acting antibiotics and affects ß-lactam resistance.

The new two-component system of *L. monocytogenes*, named VirS/VirR, which is necessary for virulence, was recently identified using signature-tagged mutagenesis (Mandin et al., 2005). A transcriptomic analysis revealed that VirS controls the expression of 108 other genes, probably by crosstalking with another response regulator, and that 12 genes are regulated by VirR, including the *dlt* operon (Mandin et al., 2004), previously shown to be important for *L. monocytogenes* virulence (Abachin et al., 2002). A conserved DNA sequence located upstream of all the transcriptional units regulated by VirR was identified by in silico analysis, probably representing the DNA binding site of VirR. The role of all sixteen putative two-component systems of *L. monocytogenes* was undertaken systematically, by the introduction of in frame deletions into 15 out of the 16 response regulator genes and the resulting mutants were characterized. With one exception, the deletion of the individual response regulator genes has only minor effects on *in vitro* and *in vivo* growth of the bacteria. The mutant carrying a deletion in the orthologue of the *Bacillus subtilis* response regulator gene *degU* showed reduced virulence in mice, indicating that DegU is involved in the regulation of virulence-associated genes (Williams et al., 2005). Knudsen and colleagues have shown that the DegU response regulator is a pleiotropic regulator involved in expression of both motility at low temperature and in vivo virulence in mice (Knudsen et al., 2004).

PrfA—Key Regulator of *Listeria monocytogenes* Virulence Genes The PrfA (positive regulatory factor A) protein of *L. monocytogenes* functions as a master regulator, which is required and directly involved in the control of the differential expression of the *L. monocytogenes* virulence genes. PrfA is a 237-amino acid protein encoded by the first gene of the virulence gene cluster of *L. monocytogenes* whose expression is under the control of this transcriptional regulator (Leimeister-Wachter et al., 1990; Mengaud et al., 1991b; Chakraborty et al., 1992; Dramsi et al., 1993). The *prfA* gene lies downstream from and is co-transcribed with *plcA-prfA* is transcribed as either a monocistronic or a bicistronic *plcA-prfA* transcript, thereby creating an autoregulatory loop essential for the appropriate expression of virulence genes (Mengaud et al., 1991b) (Fig. 44). Evidence for an overexpression of PrfA inside cells has been observed (Klarsfeld et al., 1994; Renzoni et al., 1999).

Molecular and Structural Features On the basis of structural and functional features shared with the cAMP receptor protein (Crp) of *E. coli*, PrfA is clearly a member of the Crp/Fnr (fumarate

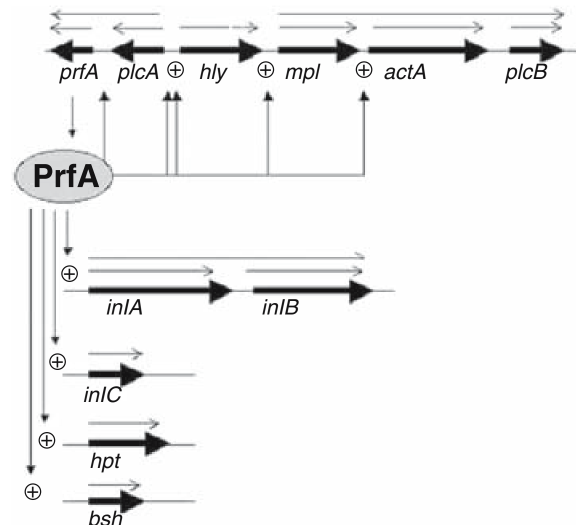

Fig. 44. The PrfA regulon in *L. monocytogenes*. *prfA*: positive regulatory factor; *plcA*: phosphatidylinositol phospholipase C; *hly*: listeriolysin O; *mpl*: metalloprotease; *actA*: actin-polymerization protein; *plcB*: broad-range phospholipase C (lecithinase); *inlA*, *inlB*: large cell wall associated internalins A and B; *inlC*: small secreted internalin, *hpt*: hexose phosphate transporter; *bsh*: bile salt hydrolase; and + indicates transcriptional induction. Thin arrows above the gene arrows indicate the different transcripts.

nitrate reductase regulator) family of transcriptional regulators (Lampidis et al., 1994). Interestingly this family of regulators seems to be of particular importance for *Listeria* insofar as there are 15 Crp/Fnr family members in *L. monocytogenes* in contrast to only one in *B. subtilis* and two in *E. coli* (Glaser et al., 2001). Figure 45 shows the domain organization of PrfA from *L. monocytogenes* compared to that of Crp from *E. coli*. In the N-terminal domain, both contain several ß-sheets delimited by glycine residues forming a ß-roll structure and an α-helical region. In Crp, both are involved in binding of the cofactor cAMP. In PrfA several of the amino acids required for cAMP binding are not conserved and the role of this ß-roll structure is not known yet. Two of the three activating regions of Crp, which mediate the interaction with the RNA polymerase, are conserved in PrfA (Fig. 45). PrfA has in its C-terminal region a DNA-binding helix-turn-helix (HTH) region with 70% similarity to the corresponding HTH in Crp. The crystal structure of PrfA has recently been solved confirming the above information (Thirumuruhan et al., 2003; Protein Data Bank (http://www.rcsb.org/pdb), accession code 1OMI; Eiting et al., 2005). Activation of genes by PrfA requires binding of this HTH region to a 14-bp palindromic sequence present at PrfA regulated promoters, the PrfA-box (Freitag et al., 1993; Bockmann et al., 1996; Sheehan et al., 1996). This palindrome is centered at position –41 relative to the transcriptional start site and partially overlaps the –35 promoter regions. It was shown that the two critical elements of PrfA-dependent promoters, the PrfA-box and the –10 box, can be functionally exchanged as long as the distance in between is maintained to 22 or 23 bp (Luo et al., 2005). PrfA activates all genes of the virulence gene cluster (*prfA*, *plcA*, *hly*, *mpl*, *actA* and *plcB*) as well as the *inlA* and *inlB* (Dramsi et al., 1997), *inlC* (Engelbrecht et al., 1996; Lingnau et al., 1996), *bsh* (Dussurget et al., 2002) and *hpt* (*uhpT*) genes (Chico-Calero et al., 2002). Table 6 shows the PrfA-box sequences of these PrfA regulated virulence genes in *L. monocytogenes*. That the PrfA box is indeed the recognition sequence for PrfA has been suggested by the effect of mutations in this sequence (Freitag et al., 1992). Further evidence came from gel retardation assays and in particular from DNAse I protection experiments (Bockmann et al., 1996). At the C-terminus, PrfA has an extension as compared to Crp with the characteristics of a leucine zipper (Lampidis et al., 1994). However, different studies indicate that the mechanisms regulating the *L. monocytogenes* virulence genes are very complex and show that PrfA is not the only regulatory factor. By using an *in vitro* transcription assay for *L. monocytogenes* genes, it was shown that overlapping PrfA-dependent and -independent promoters that are differentially activated by GTP are present for the PrfA-dependent genes *inlC* and *mpl*, indicating that these genes are not solely regulated by PrfA. Furthermore, PrfA-independent transcription of

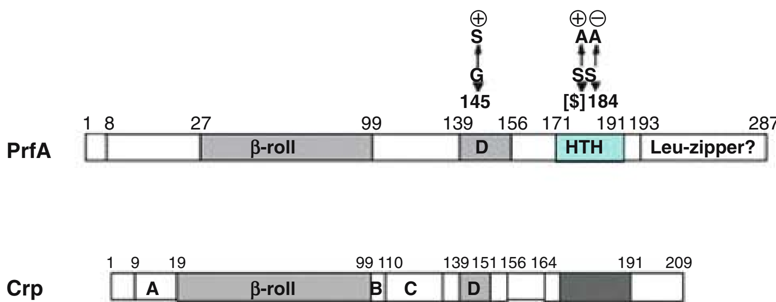

Fig. 45. Domain organization of PrfA (positive regulatory factor) from *L. monocytogenes* and Crp (cyclic AMP receptor protein) from *E. coli*. Numbers indicate start and end of domains. A-D: alpha helices in Crp, β-roll structure: cAMP binding region in Crp from *E. coli* and similar structure in PrfA. Leu-zipper?: putative leucine zipper. Adapted from Goebel et al. (2000), with permission.

Table 6. PrfA (positive regulatory factor)-box of PrfA regulated virulence genes in *L. monocytogenes*.

Promoter		PrfA-box	Spacer –10 box Startcodon
P*2hly* 5′	CA	TTAACATTTGTTAA	–N23-TAGAAT-N139-ATG-3′
P*plcA* 5′	CG	TTAACAAATGTTAA	–N22-TAAGAT-N31-TTG-3′
P*mpl* 5′	AA	TTAACAAATGTAAA	–N22-TATAAT-N156-ATG-3′
P*actA* 5′	GA	TTAACAAATGTTAG	–N21-GATATT-N157-GTG-3′
P*inlA* 5′	GG	ATAACATAAGTTAA	–N22-TATTAT-N402-GTG-3′
P*inlC* 5′	AT	TTAACGCTTGTTAA	–N22-TAACAT-N106-TTG-3′
P*hpt* 5′	TG	ATAACAAGTGTTAA	–N23-TATATT-N152-ATG-3′
P*bsh* 5′	AT	TTAAAAATTTTTAA	–N30-TATGAG-N109-ATG-3′
P*prfA* 5′	AG	CTAACAATTGTTGT	–N21-TATTTT-N37-ATG-3′
		→ ← TTAACANNTGTTAA	

inlC and *mpl* was shown to be strongly inhibited by PrfA because of the close proximity of the PrfA binding site to the –35 box (Luo et al., 2004). Shen and Higgins demonstrate that the *hly* 5′ UTR plays a critical role in regulating expression of LLO during intracellular infection (Shen and Higgins, 2005). They suggest that the hly 5′ UTR functions independently of PrfA-mediated transcription and can enhance expression of cis-associated genes through a mechanism that appears to act at both a post-transcriptional and post-translational level. Deletion of the hly 5′ UTR, while retaining the hly ribosome binding site, had a moderate effect on LLO production during growth in broth culture, yet resulted in a marked decrease in LLO levels during intracellular infection (Shen and Higgins, 2005).

Functional Changes Due to Alterations in Defined Positions Specific amino acid changes in PrfA correlates with high or low level of hemolytic activity, because of high- or low-level expression of the *hly* gene. A Gly145Ser substitution (Fig. 45) is responsible for a high hemolytic phenotype (Ripio et al., 1997b). This mutation freezes PrfA in its active conformation leading to the expression of a constitutively active PrfA* form (Ripio et al., 1996). The crystal structure of PrfA* has been solved and compared to that of wild-type PrfA, showing that the HTH motifs and adopt a conformation similar to cAMP-induced Cap, which favors DNA binding (Eiting et al., 2005). The correlation of the high hemolytic phenotype with the altered PrfA protein is also supported by the observation that the transfer of the *prfA** gene to a strain with low hemolytic activity shifts it to a strain with high hemolytic activity (Bohne et al., 1996; Ripio et al., 1997b). A Leu140Phe mutation and an Ile45Ser mutation, mapping to the N-terminal β-roll structure, also cause a constitutive overexpression of the PrfA regulon (Vega et al., 2004; Wong and Freitag, 2004). Wong and Freitag report that the mutation Leu140Phe results in the aggregation of *L. monocytogenes* in broth culture and, unlike previously described *prfA* mutations, appears to be slightly toxic to the bacteria (Wong and Freitag, 2004). As shown by Sheehan and colleagues, a Ser183Ala exchange also leads to increased binding of PrfA causing enhanced expression of virulence genes, whereas a Ser184Ala exchange leads to decreased binding of PrfA to its target sequence and thus to reduced expression of virulence genes (Sheehan et al., 1996) (Fig. 45). Shetron-Rama and colleagues selected *L. monocytogenes* mutants expressing high levels of *actA* during *in vitro* growth after chemical mutagenesis (Shetron-Rama et al., 2003). This led to the identification of two different amino acid substitutions within PrfA (Glu77Lys and Gly155Ser), which also appear to lock PrfA in its activated state. Both *prfA* Glu77Lys and *prfA* Gly155Ser strains are more efficient than wild-type bacteria in gaining access to the host cell cytosol and in initiating the polymerization of host cell actin, and both are capable of mediating LLO-independent lysis of host cell vacuoles in cell lines for which *L. monocytogenes* vacuole disruption normally requires LLO activity (Mueller and Freitag, 2005). Positive selection of mutations leading to loss or reduction of transcriptional activity of PrfA led to the identification of mutations in three regions of the PrfA protein: 1) between amino acids 58–67 in the β-roll domain, 2) between amino acid 169–193, corresponding to the DNA-binding HTH motif, and 3) in the 38 C-terminal amino acids of PrfA, which form the putative leucine-zipper-like structure. Mutations in the HTH motif and the leucine-zipper-like structure lead to PrfA proteins unable to bind to the PrfA-binding site (Herler et al., 2001).

Physicochemical Parameters Influencing PrfA A number of physicochemical parameters are known to affect the expression of PrfA-dependent genes. For example, elevated iron concentrations in the medium repress transcription of *hly* and *actA*, whereas growth in activated charcoal-containing BHI or in minimum essential medium results in induction of *prfA* and PrfA-dependent gene expression. Furthermore, the presence and utilization of different carbohydrates has a remarkable impact on the virulence of *L. monocytogenes* (Kreft and Vazquez-Boland, 2001). For instance, growth on glucose-1-phosphate (G-1-P) as sole carbon source is strictly PrfA-dependent (Ripio et al., 1997a). It was shown that the PrfA-dependent utilization of this compound is necessary for efficient cytosolic replication of *L. monocytogenes* (Chico-Calero et al., 2002). An interesting observation is that metabolizable unphosphorylated sugars inhibit the expression of PrfA-dependent virulence genes. This mechanism was first discovered with cellobiose, but later any fermentable carbohydrate was found to trigger the downregulation of PrfA-dependent virulence genes. CcpA (catabolite control protein A) was suggested to be an important element of carbon source regulation in *L. monocytogenes*, but utilizable sugars still downregulate the expression of *hly*. In a *ccpA* mutant, CcpA does not seem to be involved in carbon source regulation of virulence genes (Behari and Youngman, 1998). Interestingly, in the presence of cellobiose, PrfA is fully expressed, suggesting that PrfA is post-transcriptionally modified (Renzoni et al., 1997)

and can switch between a transcriptionally active and inactive form upon interaction with an hypothetical activating factor (Renzoni et al., 1997; Ripio et al., 1997b, Vega et al., 1998). This is also suggested by the fact that *L. monocytogenes* strains producing a constitutively active PrfA form are refractory to sugar-mediated virulence gene expression (Ripio et al., 1997a). Isolation of mutants that exhibit high levels of PrfA-controlled gene expression in the presence of cellobiose or glucose led to the identification of mutations in two different genetic loci, *gcr* and *csr*, both unlinked to the major virulence cluster. A mutation in *gcr* deregulates the expression of PrfA-controlled genes in the presence of several repressing sugars and other environmental conditions, a phenotype similar to that of a Gly145Ser substitution in PrfA itself. A mutation in the *csr* locus, within *csrA*, results in a cellobiose-specific defect in virulence gene regulation. Mutations in both *gcr* and *csr* are required for full relief of cellobiose-mediated repression of the PrfA regulon, suggesting the existence of two semi-independent pathways for cellobiose-mediated repression (Milenbachs Lukowiak et al., 2004). Expression of *prfA* and *hly* in murine macrophage-like J774.1 cells, with or without activation by IFN-γ plus LPS, showed that expression of *hly* inside activated macrophage was abolished by addition of SOD and catalase, suggesting that reactive oxygen intermediates contribute to the upregulation of *prfA* and *hly* transcriptions. Moreover, exposure of *L. monocytogenes* to H_2O_2 dramatically enhanced the expression of both *prfA* and *hly* mRNAs, suggesting that oxygen radicals generated in activated macrophages provide a positive signal for up-regulation *prfA* and the *prfA*-regulated virulence genes in *L. monocytogenes* (Makino et al., 2005).

The PrfA-regulon Analyzed at the Genome Level The PrfA-regulon and the influence of physicochemical factors on gene regulation by PrfA have been studied at the complete genome level by a transcriptomics approach (Milohanic et al., 2003; Rauch et al., 2005). Using whole-genome arrays based on the sequenced *L. monocytogenes* EGDe strain (Glaser et al., 2001), the expression profiles of two *L. monocytogenes* wild-type strains (EGDe and P14*) were compared to those of their *prfA*-deleted mutant strains. The study of three different growth conditions, rich-medium (BHI), BHI supplemented with active charcoal, and BHI supplemented with cellobiose allowed three groups of genes differently regulated by PrfA to be identified. One group included the already known virulence genes and two newly identified genes (*lmo2219* and *lmo0788*), which all contain a PrfA-box. These results indicate that the core set of genes directly regulated by PrfA is quite small (i.e., in the conditions tested, only 12 genes [*hly*, *mpl*, *actA*, *plcB*, *plcA*, *prfA*, *inlA*, *inlB*, *inlC*, *hpt*, *lmo2219*, and *lmo0788*]). Most interestingly, a second group of genes encoding a putative sugar transport system was shown to be negatively regulated by PrfA, indicating that PrfA can also act as a repressor. The most surprising result was the identification of a third group of 53 genes (group 3), which was regulated but did not contain a PrfA binding site. In depth analysis revealed sigma B like promoters in front of nearly all the genes of this group 3, suggesting interplay between PrfA and sigma B (Milohanic et al., 2003). This finding is substantiated by a report by Nadon and colleagues who reported the P_2*prfA* is a sigma B dependent promoter (Nadon et al., 2002). A transcriptome analysis comparing a wild-type and a *sigB* deleted mutant strain using focused microarrays containing 208 putative sigma B regulated genes and known *L. monocytogenes* virulence genes showed that sigma B regulates the stress response. It also confirmed the previous implication of sigma B in regulation of virulence functions (Kazmierczak et al., 2003).

Temperature Regulation of PrfA Expression Pathogenic Listeriae face a sudden increase in temperature during the transition from the environment to a warm-blooded host. This temperature shift correlates with a maximal expression of the virulence genes at 37°C but nearly no expression at 30°C. The low expression of virulence genes coincides with the absence of PrfA protein, although the *prfA* gene is still transcribed (Leimeister-Wachter et al., 1992; Renzoni et al., 1997; Johansson et al., 2002). At low temperatures, *prfA* is transcribed from its own promoter, giving rise to a monocistronic *prfA* transcript. At 37°C, transcription originates both from the *prfA* promoter and from the PrfA-dependent *plcA*-promoter (Fig. 46). However, the absence of PrfA protein at temperatures lower than 30°C is not due to a general untranslatability of the *prfA* monocistronic messenger. The mechanisms underlying thermoregulated production of PrfA have recently been elucidated. At low temperature, the untranslated mRNA region (UTR) preceding *prfA* forms a secondary structure, which masks the ribosome-binding site. At high temperature, the structure is destabilized (Fig. 47). Thus, a RNA "thermosensor" controls the expression of virulence genes in *L. monocytogenes* (Johansson et al., 2002).

Regulation through Sigma Factors L. monocytogenes is predicted to contain only five sigma factors in comparison to 18 in *B. subtilis* (Kunst

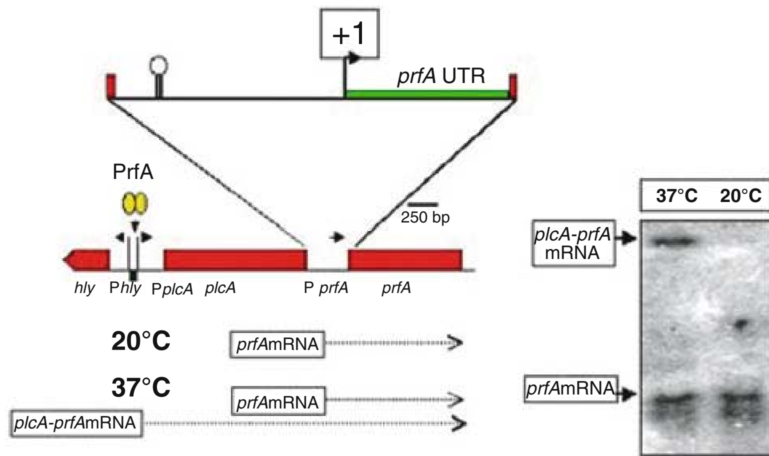

Fig. 46. Transcription of the *prfA* (positive regulatory factor) gene at both 20°C and 37°C. A) Schematic representation of *prfA* transcription. The mono- and the bicistronic mRNA at 20°C and 37°C are indicated as dotted flashes under the map. The intergenic region between *plcA* (phosphatidylinositol phospholipase C) and *prfA* are enlarged above the map indicating the transcriptional start (+1) and the *prfA*-UTR (untranslated region). B) Northern blot analysis of *prfA* transcription. Wild-type *L. monocytogenes* strain LO28 was grown to late logarithmic phase in brain–heart infusion (BHI) media at either 20° or 37°C. From Johansson et al. (2002), with permission.

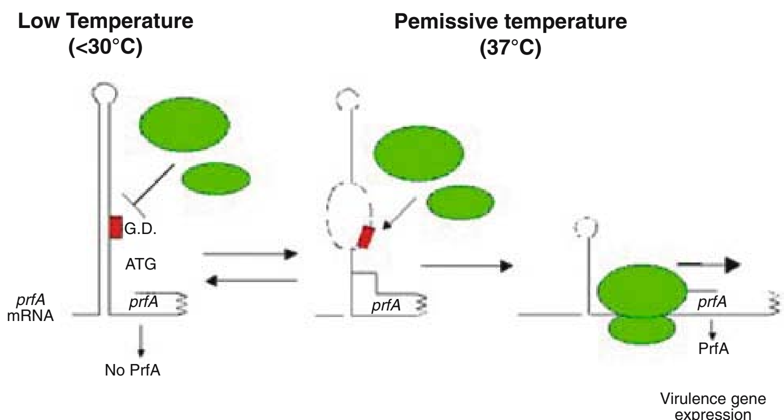

Fig. 47. Model of mechanism underlying thermoregulated expression of PrfA (positive regulatory factor). The *prfA*-UTR (untranslated region) forms a secondary structure at low temperatures (30°C) masking the ribosomal region of *prfA*, thus preventing the binding of the ribosome. PrfA is not translated and virulence genes are not expressed. At high temperatures (37°C) the *prfA*-UTR partially melts, and thereby permits binding of the ribosome to the Shine-Dalgarno sequence. From Johansson et al. (2002), with permission.

et al., 1997) and 13 in *Mycobacterium tuberculosis* (Cole et al., 1998). These are similar to *B. subtilis* SigA, SigB, SigH, SigL (RpoN, sigma 54) and ECF-type sigma factors. Sigma 54 of *L. monocytogenes* has been described to be involved in sensitivity to antibacterial peptides, the subclass IIa bacteriocins (Robichon et al., 1997). One sigma 54-dependent operon, *mptACD*, has been characterized in details in *L. monocytogenes*. Its expression is controlled by the ManR activator, which belongs to the LevR family (Dalet et al., 2001). The *mptACD* operon encodes the AB, C and D subunits of a PTS permease of the mannose family, EIItMan. The lack of *mptACD* expression, in a *mpt* or a *rpoN* mutant, leads to resistance of *L. monocytogenes* to subclass IIa bacteriocins. The EIItMan permease was thus proposed to be the receptor for these antibacterial peptides (Dalet et al., 2001; Duché et al., 2002; Gravesen et al., 2002). Study of the role of the alternative sigma 54 factor by comparing the global gene expression and the protein content of *L. monocytogenes* strain EGDe suggested that sigma 54 is mainly involved in the control of carbohydrate metabolism in *L. monocytogenes* via direct regulation of the PTS activity, alteration of the pyruvate pool and modulation of carbon catabolite regulation (Arous et al., 2004).

The alternative sigma factor sigma B modulates the stress response of several Gram-positive bacteria, including *Bacillus subtilis* and the food-borne human pathogens *Bacillus cereus*, *L. monocytogenes* and *Staphylococcus aureus*. In all these bacteria, sigma B is responsible for the transcription of genes that can confer protection to the cell against adverse conditions. Upon exposure to stress, the stress has to be sensed and signaled through a regulatory cascade, leading to the activation of sigma B and, subsequently, to

the transcription of the set of sigma B-regulated genes (the Sigma B regulon). The encoded proteins perform specific functions, which protect the cell against stress. In *L. monocytogenes*, sigma B was shown to have a role in growth and survival under low temperatures, acid tolerance, survival under environmental, energy, and intracellular stress conditions (Becker et al., 2000; Wiedmann et al., 1998; Chaturongakul and Boor, 2004). This condition is of special interest for practical reasons, as chilled storage is often a crucial factor in the preservation of minimally processed foods. The deletion of *sigB* had only very minor effects on virulence, as tested in animals models (Wiedmann et al., 1998), even though several virulence factors in this organism are under the control of sigma B (see below). The *bsh* gene encoding the bile salt hydrolase and two genes from the internalin family, which contribute to bacterial entry are partially sigma B-dependent and also contribute to PrfA-mediated virulence in *Listeria monocytogenes* (Kazmierczak et al., 2003; Sue et al., 2003; Nadon et al., 2002; Milohanic et al., 2003). Furthermore, the central role of sigma B in the regulatory network is illustrated by the fact that sigma B can control other important regulatory proteins like the RNA-binding protein Hfq in *L. monocytogenes*. Hfq is thought to play a crucial role in the post-transcriptional regulation of gene expression by small RNAs and the dependency of this regulator on sigma B adds another level of complexity to the function of sigma B in *L. monocytogenes* (Christiansen et al., 2004).

Animal Models

Considerations for the Choice of an Adequate Animal Model An infection is a multistep process integrating a number of host and microbial variables. For this reason, comprehension of the pathophysiology of a human infection necessitates an animal model in which the infectious agent has the same cell and tissue tropism as in humans. Similarly, an animal model should allow observation of the same direct effects and indirect immunopathological damages occurring in humans. Such a model should allow testing the in vivo relevance of results acquired using more reductionist in vitro approaches. Ideally, both the microbial pathogen and the animal model should be genetically amenable, thus allowing assessment of the role of critical microbial and host factors during the infectious process. The genetic manipulation of both the pathogen and the host may lead to a better understanding of their respective contributions in the complex interplay that results in disease (Finlay, 1999; Harvill and Miller, 2000; Lecuit and Cossart, 2002).

Since animal listeriosis exists, animal species naturally infected by *L. monocytogenes* can be used as models to study the pathophysiology of human listeriosis. However, this also implies important technical limitations: the animals developing a disease closely resembling human listeriosis are not classical laboratory animals such as the rat or the mouse but rather farm animals such as sheep, cattle and goats. As for other human diseases, the murine model became the most widely used animal model in mammal biology. Indeed, mice have all the necessary qualities to make them good laboratory animals: smallness, resistance and adaptation to captivity, easy reproduction, abundant offspring, short gestation period, and a physiology and pathophysiology in most respects comparable to humans. However, certain human pathogens are not mouse pathogens, and mouse disease induced by human pathogens can be manifestly different from the actual human disease. With this in mind, the mouse is frequently regarded as an unsatisfactory model for the study of human infectious diseases, which still continues to be used in the absence of better models (Lecuit and Cossart, 2002).

A review of the literature did not find reports of symptomatic natural *L. monocytogenes* infection in mice and rats. Experimentally, the mouse, like the rat, which is phylogenetically extremely close to the mouse species, cannot be easily infected with *L. monocytogenes* by the oral route. Most studies using mice or rats fail to induce a reproducible lethal infection after oral infection with *L. monocytogenes*, even using extremely high inocula (Zachar and Savage, 1979; Roll and Czuprynski, 1990; Okamoto et al., 1994; Gaillard et al., 1996; MacDonald and Carter, 1996; Marco et al., 1997; Pron et al., 1998; Huleatt et al., 2001; Manohar et al., 2001). This implies that one has to consider with caution results of in vivo studies using dose-response mathematical models to determine the theoretical lethal dose in humans after oral ingestion of contaminated food (Haas and Thayyar-Madabusi, 1999).

To circumvent the low susceptibility of mice infected via the oral route, alternate routes of administration were used, although they do not mimic the natural route for infection. They include intravenous and intraperitoneal, or more rarely intranasal, subcutaneous, conjunctival, intracardiac or intracerebral routes, not only in mice but also in guinea pigs, rabbits and sheep (Kautter et al., 1963; Racz et al., 1970; Khan et al., 1972; Dustoor et al., 1977; Scheld et al., 1979; Prats et al., 1992). In mice, inoculation via the intravenous rather than the oral route can produce a lethal infection, and thus allows the determination of the LD_{50} and comparison of the virulence of various mutants. This route of

administration was particularly instrumental in characterization of most *L monocytogenes* virulence genes (Gaillard et al., 1986; Kathariou et al., 1987; Cossart et al., 1989b; Domann et al., 1992; Kocks et al., 1992; Raveneau et al., 1992). It also allowed demonstrating that immunosuppression, young age and gravidity were associated with a reduced LD_{50}. Finally, it revealed granulomatous hepatitis due to intravenously-acquired murine listeriosis was similar to that observed in *granulomatosis infantiseptica* (Luft and Remington, 1982; Stelma et al., 1987; Genovese et al., 1999). However, this symptomatic septicemic infection seems different from what is observed in the human species, for which the phase preceding the feto-placental or CNS infection is mostly clinically silent.

Infection by the intravenous route in the mouse model also allowed the discovery of cellular immunity and its cellular basis (CD8+ lymphocytes) (Mackaness, 1962; Mackaness and Hill, 1969; North, 1969; Pearson and Osebold, 1973; Kaufmann, 1988). The pioneering studies carried out by Mackaness in the 1960s made *L. monocytogenes* one of the best-characterized and most instrumental models of intracellular microbes (Mackaness, 1962; Mackaness and Hill, 1969). However, the low capacity of *L. monocytogenes* to infect mice via the oral route has limited the use of *L. monocytogenes* to the field of systemic immunity at the expense of mucosal immunity. This model of infection also made it possible to demonstrate that genetic background played a part in the susceptibility to *L. monocytogenes*, since BALB/c mice are more sensitive to *L. monocytogenes* than C57 BL/6 following intravenous inoculation (Cheers and McKenzie, 1978). The molecular mechanisms underlying these susceptibility differences are unknown. Their identification should lead to a better understanding of the human inter-individual variation in susceptibility to *L. monocytogenes* (Boyartchuk et al., 2001).

Addressing the Species-Specificity of *L. monocytogenes* in Animal Models A number of human pathogens exhibit stringent host specificity. For some of them, the molecular basis of their restricted host-tropism has been at least partially deciphered. It implicates the species-specific interaction of a microbial ligand with its cellular receptor(s), as demonstrated for viruses such as the poliovirus, the measles virus, HIV and hepatitis C virus, but also for bacteria such as *Neisseria gonorrhea* and *Neisseria meningitidis*, and more recently *L. monocytogenes* (Dalgleish et al., 1984; Mendelsohn et al., 1989; Dorig et al., 1993; Alkhatib et al., 1996; Dragic et al., 1996; Virji et al., 1996; Chen et al., 1997; Gray-Owen et al., 1997; Pileri et al., 1998; Lecuit et al., 1999; Lecuit et al., 2001b). Study of a species-specific ligand-receptor interaction, thought to play a critical role in human infection, should be possible in an animal model that allows this interaction to occur. Two possibilities exist to establish such a model: the simplest one is to identify an animal species in which the interaction does occur (such as cattle, sheep and goats for *L. monocytogenes*) and to use it as a model. A more sophisticated approach is to generate a genetically modified animal, generally a transgenic mouse line that expresses the human receptor and to look at the effect of the transgene expression on the infectious process. The human receptor can be expressed either in place of or in addition to its nonfunctional mouse orthologue, either ubiquitously or under the control of a tissue-specific promoter that drives expression of the transgene in the cell types targeted by the pathogen during the human infection (Lecuit and Cossart, 2002).

As mentioned above, in mice, oral inoculation is a very inefficient way to trigger systemic listeriosis, because *L. monocytogenes* translocation across the intestinal barrier is low and not higher than that of the closely related nonpathogenic species *L. innocua*. The few detectable foci of bacterial multiplication are restricted to Peyer's patches, areas that contain M-cells, i.e., cells possessing a phagocytic activity. Thus, *L. monocytogenes* is not an enteropathogen for mice. Moreover, in mice, the brainstem and the feto-placental unit do not appear as elective targets, as is the case in humans (M. Lecuit, unpublished results).

Following mouse intravenous inoculation, LLO, ActA, PLC-A, and PLC-B but not internalin act as virulence factors. Indeed, despite the well-established prominent role of internalin in the internalization process in vitro, its role in vivo has long remained elusive since an internalin mutant behaves as its wild-type parent after mouse intravenous or oral inoculation. Strikingly, shortly after the discovery of human E-cadherin as the internalin receptor, it was established that, in contrast to human E-cadherin, mouse E-cadherin is unable to promote entry of *L. monocytogenes* into cells. This specificity was shown to depend on a single amino acid of the mature E-cadherin peptidic chain, the sixteenth, which is proline in humans and glutamic acid in mice. This result led to the conclusion that *L. monocytogenes* exhibits a species-specificity towards its host, and that the mouse model is inappropriate to study internalin function (Lecuit et al., 1999). Similarly, InlB was shown to be species-specific being able to promote entry and ruffling in human and mice cells but not in guinea pig and rabbit cells. As a consequence, no role could be demonstrated for

InlB in guinea pigs and rabbit infections although it played a role in liver and spleen colonization in mice (Khelef et al., in press).

A more appropriate animal species for studying the putative role of internalin interaction with E-cadherin in vivo turned out to be the guinea pig. Indeed, internalin-dependent entry of *L. monocytogenes* was demonstrated in cultured guinea pig cells expressing E-cadherin, harbouring a proline at position 16 as human E-cadherin (Lecuit et al., 1999). Moreover, induction of a gastroenteritis by *L. monocytogenes* resembling that observed in humans was observed in the guinea pig (Racz et al., 1972; Dalton et al., 1997; Pron et al., 1998; Aureli et al., 2000; Lecuit et al., 2001b). In addition, it is able to cross the intestinal barrier and induce a dose- and internalin-dependent lethality following dissemination into the systemic circulation (Lecuit et al., 2001b). Yet, this model remains inappropriate to study concomitantly the role of both InlA and InlB. To investigate the role of the internalin-E-cadherin interaction in the ability of *L. monocytogenes* to cross the intestinal barrier, a transgenic mouse model was generated. The human E-cadherin cDNA was placed under the control of the promoter of the intestinal fatty acid binding protein (iFABP) gene, which is exclusively active in post-mitotic nonproliferative small intestinal enterocytes. In this transgenic model, *L. monocytogenes* directly targets enterocytes by interacting with enterocytic E-cadherin. This interaction leads to *L. monocytogenes* internalization into these cells and allows the subsequent crossing of the intestinal barrier, followed by bacterial multiplication in the small intestine lamina propria, and dissemination to mesenteric lymph nodes, liver and spleen (Fig. 48). This is the first transgenic model to reveal the role of a bacterial virulence factor and to demonstrate its critical implication in a key step of an infection process. The enterocyte-restricted human E-cadherin expression has been critical to demonstrating at a molecular level the direct in vivo targeting of enterocytes by *L. monocytogenes* and the genuine enteropathogenicity of *L. monocytogenes*. Furthermore, this transgenic model not only provides the molecular explanation for the innocuity of *L. monocytogenes* in mice following oral infection but also explains the enteropathogenicity of *L. monocytogenes* in guinea pigs, and most probably in humans (Lecuit et al., 2001b). A mouse model for orally acquired listeriosis is now available. The host response to listeriosis can now be studied in depth from its starting point (the intestinal lumen) using the combined approaches of microbial genetics, transgenesis, gnotobiology, and functional genomics, coupled to laser capture microdissection, as described in the study of host responses to commensal bacteria by Hooper and Gordon (2001). The availability of the *L. monocytogenes* genome, together with that of its nonpathogenic counterpart *L. innocua*, will probably be very helpful in identifying additional virulence factors in this new model. This system, however, as the models for human viral infections presented above, has its limits. These were anticipated when the transgenesis strategy was designed. Indeed, since human E-cadherin expression is restricted to enterocytes in the *iFABP*-human E-cadherin transgenic mice, the role of other cell types expressing E-cadherin (such as dendritic cells, hepatocytes, microvascular endothelial cells, epithelial cells of the choroid plexus, and cytotrophoblastic cells, all of which are putative *L. monocytogenes* targets during human listeriosis) cannot be addressed. Mice overcoming this limitation are now being generated by changing the codon for glutamic acid 16 of mouse E-cadherin at the endogenous mouse E-cadherin locus *Cdh1* on chromosome 8 into a codon for proline. This unique change in murine E-cadherin has indeed been shown to be sufficient to convert mouse E-cadherin into an internalin receptor in transfected cultured cells expressing this modified Glu16Pro mouse E-cadherin (Lecuit et al., 1999). The new transgenic mouse line should permit the study of the tropism of *L. monocytogenes* for the CNS and the feto-placental unit (which is responsible for the lethality of human listeriosis) and to further investigate the role of internalin E-cadherin interaction in extraintestinal tissues. This model

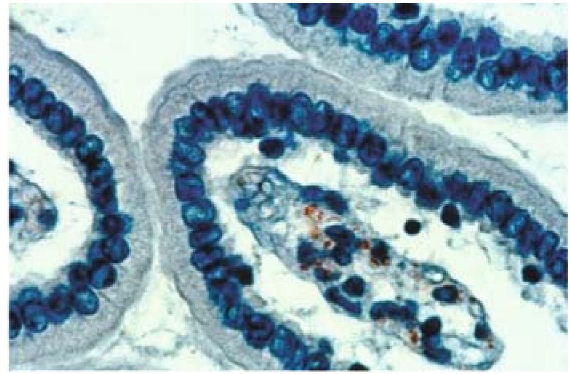

Fig. 48. *Listeria monocytogenes* multiplication within the lamina propria of a small intestinal villus of an *iFABP-hEcad* (intestinal fatty acid binding protein-human E-cadherin) transgenic mouse 48 h after intragastric inoculation. A section through the small intestine of an *iFABP-hEcad* transgenic mouse is immunolabeled with rabbit anti-*L. monocytogenes* R11 polyclonal antibody and the *Listeria* are revealed with anti-peroxidase antibodies (red). *Listeria monocytogenes* have crossed the intestinal barrier and replicate in the lamina propria, a phenomenon that is never observed in nontransgenic mice. Scale bar 10 μm. From Lecuit et al. (2001b), with permission.

should also allow addressing the role of InlB in mice after oral infections. The importance of these future studies is emphasized by the results of our recent epidemiological investigations, which showed that 100% of *L. monocytogenes* isolates obtained from pregnancy-associated listeriosis and collected in a one-year period express a functional internalin, whereas only 65% of food isolates collected during the same period express functional internalin. These results strongly argue in favor of a role for internalin in crossing the maternofetal barrier (Jacquet et al., 2004). This hypothesis has now been confirmed experimentally (Lecuit et al., 2004). Recent studies using different *L. monocytogenes* strains reveal that, in contrast to strains from other serovars, serotype 4b epidemic strains appear to be able to cause systemic infection in mice infected orally. This suggests that serovar-specific virulence factors might be playing a role in mouse susceptibility to orally acquired listeriosis. These observations highlight that the properties of an animal model may vary from strain to strain of a single bacterial species (Czuprynski et al., 2003).

Comparative Genomics

Comparative genomics and related technologies are helping to unravel the molecular basis of pathogenesis, host range, evolution and phenotypic differences of Listeriae. The first application of genomics to *Listeria* research was the determination by a consortium of 10 European laboratories of the complete genome sequences of the pathogen *L. monocytogenes* (strain EGDe) and the closely related nonpathogenic species *L. innocua* (strain CLIP11626) (Glaser et al., 2001). The availability of these two sequences (see ListiList Worldwide Web Server (http://genolist.pasteur.fr/ListiList/)) opens the way to comparative and functional genomics and the use of new approaches like bioinformatics, microarrays and proteomics to gain functional information (Fig. 49). Additional sequence information is available as a second *L. monocytogenes* isolate, strain F2365 (Scott A), is being sequenced by the TIGR {Technical Institute of Genomic Research} in collaboration with the USDA. Furthermore, the determination of the genome sequences of *L. ivanovii*, *L. seeligeri*, *L. welshimeri* and *L. grayi* strains by the German PathoGenomik network in collaboration with the Institut Pasteur are nearing completion. The availability of these different genome sequences of Listeriae will pave the way for in-depth comparative genomics and the identification of unknown virulence determinants.

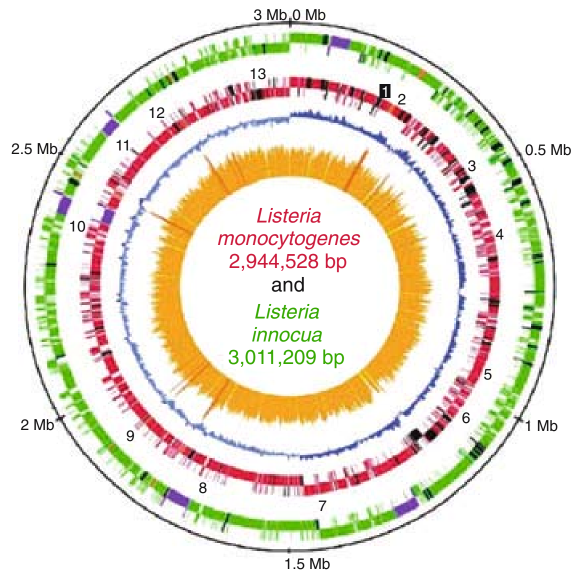

Fig. 49. Circular genome maps of *L. monocytogenes* EGD-e and *L. innocua* CLIP 11262 showing the position and orientation of genes. Circle 1 and 2, *L. innocua* and *L. monocytogenes* genes on the + and – strands, respectively. *Listeria innocua* genes, green; *L. monocytogenes* genes, red; genes specific for *L. monocytogenes* or *L. innocua*, black; rRNA operons, orange; and prophages, purple. Numbers on the second circle indicate the position of known virulence genes: 1, virulence locus (*prfA-plcA-hly-mpl-actA-plcB*); 2, *clpC*; 3, *inlAB*; 4, *iap*; 5, *dal*; 6, *clpE*; 7, *lisRK*; 8, *dat*; 9, *inlC*; 10, *arpJ*; 11, *clpP*; 12, *ami*; and 13, *bvrABC*. Circle 3, G/C bias (G1C/G–C) of *L. monocytogenes*. Circle 4, G1C content of *L. monocytogenes* (32.5% G1C in light yellow, 32.5–43.5% in yellow, and >43.5% G1C in dark yellow). The scale in megabases is indicated on the outside of the genome circles, with the origin of replication at position 0. From Glaser et al. (2001), with permission.

THE GENOME SEQUENCE OF *L. MONOCYTOGENES* EGDE AND *L. INNOCUA* CLIP11262 The *L. monocytogenes* EGDe genome comprises 2,944,528 bp with an average G+C content of 39% and 2853 predicted protein-coding genes. The *L. innocua* CLIP11626 chromosome is 3,011,209 bp long with an average G+C content of 37% and 2981 predicted protein-coding genes (Glaser et al., 2001). Analysis of the two *Listeria* genomes allowed common and particular features of Listeriae to be determined, as well as differences between a pathogenic and a nonpathogenic *Listeria* strain. One interesting common feature is the finding that 2587 of the 2853 *L. monocytogenes* genes have an orthologue in the *L. innocua* genome (Glaser et al., 2001). Furthermore, a perfect conservation of the order as well as the relative orientation of these orthologous genes was identified, indicating a high stability in the genome organization of Listeriae and a close phylogenetic relationship of the two *Listeria* genomes (Buchrieser et al., 2003). However, despite this high number of common genes, considerable differences in gene content exist

between the two *Listeria* genomes, some of which are undoubtedly related to the ability of *L. monocytogenes* to cause disease in humans and animals.

A particular feature of the *Listeria* genomes is the presence of an exceptionally large number of surface proteins, as 4.8% of all predicted genes of *L. monocytogenes* EGDe and 4.3% of the *L. innocua* genome code for surface proteins. Interestingly, the differences between the two *Listeria* genomes are also the most pronounced within genes encoding surface proteins, in particular among those that encode proteins possessing a peptidoglycan anchoring domain (LPXTG motif) (Navarre and Schneewind, 1999). The *L. monocytogenes* EGDe genome encodes 41 such proteins, 19 of which are absent from the *L. innocua* CLIP11626 genome. In contrast *L. innocua* contains 34 LPXTG proteins, 14 of which are *L. innocua* CLIP11626 specific (Glaser et al., 2001; Cabanes et al., 2002). The difference in surface proteins may be related to the additional capacity of *L. monocytogenes* to interact with various eukaryotic cell types.

Besides surface proteins, an abundance of transport proteins (331 genes in *L. monocytogenes* EGDe and 313 in *L. innocua* CLIP11626) and an extensive regulatory repertoire (209 genes in the *L. monocytogenes* EGDe and 203 in *L. innocua*) are characteristic for the *Listeria* genomes. These data correlate with the capacity of *Listeria* to adapt and respond to a wide variety of different environments and to its property to colonize a broad range of ecosystems. A high percentage of the encoding capacity of the two *Listeria* genomes is dedicated to transport proteins (11.6% and 11.4%, respectively) and to regulatory proteins (7.3% and 7.1%, respectively). However, the differences between the two *Listeria* genomes are not as pronounced as the differences identified in the surface protein repertoire, suggesting their implication primarily in specific features common to the lifestyle of Listeriae outside a mammalian host.

Species-Specific Genes and Strain-Specific Genes: A Global View The genome comparison of *L. monocytogenes* EGDe and *L. innocua* CLIP11626 identified a conserved genome organization and a high number of orthologous genes, but it also revealed the presence of 270 *L. monocytogenes* EGDe-specific genes (9.5%) and 149 *L. innocua* CLIP11626-specific genes (5%), when prophages were not taken into account. The *L. monocytogenes* EGDe-specific genes are present in 100 DNA fragments scattered throughout the entire chromosome, and the *L. innocua* CLIP11626 specific genes are clustered in 63 regions containing 1–7 genes (Glaser et al., 2001; Buchrieser et al., 2003). This particular organization of a number of small regions within the *Listeria* genomes suggests that multiple acquisition, but also deletion events, have led to the present genome content. The question arises now whether these genes (e.g., the 41 genes of *L. monocytogenes* EGDe encoding for surface proteins with a LPXTG motif) are consistently present in all *L. monocytogenes* isolates or whether further differences exist among the different *L. monocytogenes* strains. To this end additional sequence information and comparative genomic approaches using the DNA-array technique were employed. *Listeria monocytogenes* strains belonging to different sub-groups other than the EGDe strain (serovar 1/2a), e.g., serovar 4b strains, exhibit considerable differences in their gene content. Indeed, about 8% of the genes are specific to this group of strains but absent from *L. monocytogenes* serovar 1/2a (Doumith et al., 2004a). These results are in line with a previous report (Herd and Kocks, 2001) that identified 39 specific gene fragments for the epidemic *L. monocytogenes* strain F4565 as compared to *L. monocytogenes* EGDe using a subtractive hybridization method. When comparing genes encoding for different protein families among the three *Listeria* strains sequenced (*L. monocytogenes* EGDe, *L. monocytogenes* serovar 4b, and *L. innocua* CLIP11262), the importance of the surface protein encoding genes, in particular those with a LPXTG anchor motif, was clearly underlined. Genes encoding proteins belonging to the LPXTG family showed the most pronounced differences among the strains. We identified a core gene pool of 20 genes encoding for LPXTG proteins and specific ones in each strain. This indicates that this protein family might be strongly implicated in strain-specific and species-specific features of Listeriae. These findings were further substantiated by the results obtained from DNA-DNA hybridization of 93 *L. monocytogenes* strains and 20 strains belonging to the remaining five species of the genus *Listeria* using DNA-arrays (Doumith et al., 2004a). Again, the distribution of surface-protein encoding genes was very heterogeneous. However, each sub-group within the species *L. monocytogenes* was characterized by the presence of specific surface protein-coding genes and only a small group is consistently present in all the *L. monocytogenes* isolates tested. For instance, differences among genes encoding phosphotransferase system (PTS) components or regulatory proteins were much less pronounced, suggesting that they account less for intra- or inter-species differences (Doumith et al., 2004a). These results further provide an explanation why previous studies have found an association between various characteristics of *L. monocytogenes* and serovars, leading to the def-

inition of three lineages within the species *L. monocytogenes*, which are correlated with serovars, suggesting that this association is due to an evolutionary differentiation.

Analysis of the hybridization profiles of different strains revealed distinct patterns of gene presence and absence in different subgroups of *L. monocytogenes* and also allowed identification of 30 *L. monocytogenes*-specific and several serovar-specific marker genes (Doumith et al., 2004a). Based on these results, four serovar-specific marker genes were selected and exploited to design a multiplex PCR, allowing the differentiation of the major *Listeria monocytogenes* serovars (Doumith et al., 2004b). These patterns and markers should further prove to be powerful tools for the rapid tracing of listeriosis outbreaks by PCR for instance, but they also provide a basis for the functional study of virulence differences between *L. monocytogenes* strains. These marker genes and the specific primers are available at Website of Genomic of Microbial Pathogens Unit at Institut Pasteur (http://www.pasteur.fr/recherche/unites/gmp/sitegmp/biodiversitylist. html).

A similar approach using a shotgun microarray and DNA-DNA hybridization corroborated these results by identifying genes specific for *L. monocytogenes* serovar 1/2a as compared to *L. monocytogenes* serovar 4b and 1/2b strains (Zhang et al., 2003). Based on *prf*A virulence gene cluster sequences from 113 *L. monocytogenes* isolates, the presence of three evolutionary lineages among the species *L. monocytogenes* was confirmed and it was shown that the human epidemic associated serotype 4b is prevalent among strains from lineage 1 and lineage 3 (Ward et al., 2004). These results also allowed to develop a PCR-based test for lineage identification.

These different genomic approaches applied to the genus *Listeria* provide for the first time a global view and a more complete knowledge of the gene distribution and the genetic content present in the gene-pool of the genus *Listeria*. This information represents a fundamental basis for functional studies to better understand phenotypic and virulence differences between *L. monocytogenes* strains.

Unsolved Questions and Concluding Remarks

In nearly two decades, *Listeria* has become one of the best-documented intracellular bacteria. Our knowledge of the bacterial factors contributing to infection is increasing daily. Yet understanding the function of the many factors encoded by the genome, involved or not in virulence, will require years of work. When are they expressed, to which regulators are they subjected, when are they active? What is the function of the intergenic regions? Are there some small RNAs involved in virulence? All these questions will require the help of system biology and large-scale genomic analysis.

Several groups are also now addressing biodiversity issues. It is clear that we do not have yet an answer to the question of why some strains are more epidemic than others. However, this information should be available soon, and genes and factors absolutely required for human infection, in addition to the well-established virulence markers LLO and ActA, should be identified soon and fully characterized. Information will also come from large epidemiological studies such as those showing that a complete and functional internalin is as critical as LLO for pathogenesis in humans (Jacquet et al., 2004; Rousseaux, 2004). More interestingly, all serovar 4b strains have a functional internalin, which is not the case for strains of other serovars (Jacquet et al., 2004).

A point that remains unclear is what the infective dose for humans? This is unknown and obviously depends on many individual factors and in particular on the immune status of the host. While ongoing research on adaptive immunity to *Listeria* infections is highly documented (Lara-Tejero and Pamer, 2004), efforts to understand the innate response to *Listeria* are now a priority.

Progress was accomplished to understand how *Listeria* cross barriers. We now know that internalin is used to cross both the intestinal and placental barriers. How does *Listeria* cross the blood brain barrier? There are arguments to predict that internalin may also be used, but the ad hoc animal model is not yet available.

Listeria is one of the "stars" in cellular microbiology (Cossart and Sansonetti, 2004). Its behavior, when in contact with the cell or when spreading intra- and intercellularly, is one of the most sophisticated bacterial behaviors. Many cellular factors contributing to infection have been identified. It is clear that what is lacking now is the spatiotemporal description of all these events at the cellular level, as well as in vivo. All the new in vivo imaging techniques are there. We just need to use them!

In conclusion, it is good that this chapter can be electronically corrected. We are at a period where information is constantly changing, and we still have a lot to learn by studying this amazing organism.

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Literature Cited

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

Alkhatib, G., C. Combadiere, C. C. Broder, Y. Feng, P. E. Kennedy, P. M. Murphy, and E. A. Berger. 1996. CC CKR5: A RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 272:1955–1958.

Allerberger, F. 2003. Listeria: Growth, phenotypic differentiation and molecular microbiology. FEMS Immunol. Med. Microbiol. 35:183–189.

Alouf, J. E. 1999. Introduction to the family of the antigenically-related cholesterol-binding ("sulfhydryl-activated") cytolytic toxins. *In:* J. E. A. Freers, a. J. H. (Eds.) The Comprehensive Sourcebook of Bacterial Protein Toxins. Academic Press. San Diego, CA. 147–186.

Alvarez-Dominguez, C., R. Roberts, and P. D. Stahl. 1997a. Internalized Listeria monocytogenes modulates intracellular trafficking and delays maturation of the phagosome. J. Cell Sci. 110:731–743.

Alvarez-Dominguez, C., J. A. Vazquez-Boland, E. Carrasco-Marin, P. Lopez-Mato, and F. Leyva-Cobian. 1997b. 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.

Annous, B. A., L. A. Becker, D. O. Bayles, D. P. Labeda, and B. J. Wilkinson. 1997. Critical role of anteiso-C15:0 fatty acid in the growth of Listeria monocytogenes at low temperatures. Appl. Environ. Microbiol. 63:3887–3894.

Archambaud, C., E. Gouin, J. Pizarro-Cerda, P. Cossart, and O. Dussurget. 2004. Translation elongation factor EF-Tu is a target for Stp, a serine-threonine phosphatase involved in virulence of Listeria monocytogenes. Molec. Microbiol. 56(2):383–396.

Archibald, A., I. Hancock, and C. Harwood. 1993. Cell wall structure, synthesis, and turnover. *In:* H. A. Losick (Ed.) Bacillus subtilis and Other Gram-positive Bacteria. ASM Press. Washington, DC. 342:1236–1241.

Armstrong, R. W., and P. C. Fung. 1993. Brainstem encephalitis (rhombencephalitis) due to Listeria monocytogenes: Case report and review. Clin. Infect. Dis. 16:689–702.

Arnaud, M., A. Chastanet, and M. Debarbouille. 2004. New vector for efficient allelic replacement in naturally non-transformable, low-GC-content, Gram-positive bacteria. Appl. Environ. Microbiol. 70:6887–6891.

Arnold, R., and W. Konig. 1998. Interleukin-8 release from human neutrophils after phagocytosis of Listeria monocytogenes and Yersinia enterocolitica. J. Med. Microbiol. 47:55–62.

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

Aureli, P., G. C. Fiorucci, D. Caroli, G. Marchiaro, O. Novara, L. Leone, and S. Salmaso. 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by Listeria monocytogenes. N. Engl. J. Med. 342:1236–1241.

Autret, N., I. Dubail, P. Trieu-Cuot, P. Berche, and A. Charbit. 2001. Identification of new genes involved in the virulence of Listeria monocytogenes by signature-tagged transposon mutagenesis. Infect. Immun. 69:2054–2065.

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

Badovinac, V. P., K. A. Messingham, S. E. Hamilton, and J. T. Harty. 2003. Regulation of CD8+ T cells undergoing primary and secondary responses to infection in the same host. J. Immunol. 170:4933–4942.

Bakardjiev, A. I., B. A. Stacy, S. J. Fisher, and D. A. Portnoy. 2004. Listeriosis in the pregnant guinea pig: A model of vertical transmission. Infect. Immun. 72:489–497.

Bannam, T., and H. Goldfine. 1999. Mutagenesis of active-site histidines of Listeria monocytogenes phosphatidylinositol-specific phospholipase C: Effects on enzyme activity and biological function. Infect. Immun. 67:182–186.

Barry, R. A., H. G. Bouwer, D. A. Portnoy, and D. J. Hinrichs. 1992. Pathogenicity and immunogenicity of Listeria monocytogenes small-plaque mutants defective for intracellular growth and cell-to-cell spread. Infect. Immun. 60:1625–1632.

Barton, G. M., and R. Medzhitov. 2003. Toll-like receptor signaling pathways. Science 300:1524–1525.

Bear, J. E., T. M. Svitkina, M. Krause, D. A. Schafer, J. J. Loureiro, G. A. Strasser, I. V. Maly, O. Y. Chaga, J. A. Cooper, G. G. Borisy, and F. B. Gertler. 2002. Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. Cell 109: 509–521.

Beauregard, K. E., K. D. Lee, R. J. Collier, and J. A. Swanson. 1997. pH-dependent perforation of macrophage phagosomes by listeriolysin O from Listeria monocytogenes. J. Exp. Med. 186:1159–1163.

Becker, L. A., M. S. Cetin, R. W. Hutkins, and A. K. Benson. 1998. Identification of the gene encoding the alternative sigma factor sigma B from Listeria monocytogenes and its role in osmotolerance. J. Bacteriol. 180:4547–4554.

Becker, L. A., S. N. Evans, R. W. Hutkins, and A. K. Benson. 2000. Role of sigma(B) in adaptation of Listeria monocytogenes to growth at low temperature. J. Bacteriol. 182:7083–7087.

Begley, M., C. G. Gahan, and C. Hill. 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., C. Hill, and C. G. Gahan. 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.

Behari, J., and P. Youngman. 1998. A homolog of CcpA mediates catabolite control in Listeria monocytogenes but not carbon source regulation of virulence genes. J. Bacteriol. 180:6316–6324.

Berche, P., J. L. Gaillard, C. Geoffroy, and J. E. Alouf. 1987a. T cell recognition of listeriolysin O is induced during infection with Listeria monocytogenes. J. Immunol. 139:3813–3821.

Berche, P., J. L. Gaillard, and P. J. Sansonetti. 1987b. Intracellular growth of Listeria monocytogenes as a prereq-

uisite for in vivo induction of T cell-mediated immunity. J. Immunol. 138:2266–2271.

Berche, P., J. L. Gaillard, and S. Richard. 1988. Invasiveness and intracellular growth of Listeria monocytogenes. Infection 16:S145–S148.

Berche, P., K. A. Reich, M. Bonnichon, J. L. Beretti, C. Geoffroy, J. Raveneau, P. Cossart, J. L. Gaillard, P. Geslin, H. Kreis, and et al. 1990. Detection of anti-listeriolysin O for serodiagnosis of human listeriosis. Lancet 335:624–627.

Bergmann, B., D. Raffelsbauer, M. Kuhn, M. Goetz, S. Hom, and W. Goebel. 2002. InlA- but not InlB-mediated internalization of Listeria monocytogenes by non-phagocytic mammalian cells needs the support of other internalins. Molec. Microbiol. 43:557–570.

Bernheim-Groswasser, A., S. Wiesner, R. M. Golsteyn, M. F. Carlier, and C. Sykes. 2002. The dynamics of actin-based motility depend on surface parameters. Nature 417:308–311.

Best, M., M. E. Kennedy, and F. Coates. 1990. Efficacy of a variety of disinfectants against Listeria species. Appl. Environ. Microbiol. 56:377–380.

Bhakdi, S., T. Klonisch, P. Nuber, and W. Fischer. 1991. Stimulation of monokine production by lipoteichoic acids. Infect. Immun. 59:4614–4620.

Bielecki, J., P. Youngman, P. Connelly, and D. A. Portnoy. 1990. Bacillus subtilis expressing a haemolysin gene from Listeria monocytogenes can grow in mammalian cells. Nature 345:175–176.

Bierne, H., S. Dramsi, M. P. Gratacap, C. Randriamampita, G. Carpenter, B. Payrastre, and P. Cossart. 2000. The invasion protein InIB from Listeria monocytogenes activates PLC-γ downstream from PI 3-kinase. Cell. Microbiol. 2:465–476.

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

Bierne, H., and P. Cossart. 2002a. InlB, a surface protein of Listeria monocytogenes that behaves as an invasin and a growth factor. J. Cell Sci. 115:3357–3367.

Bierne, H., S. K. Mazmanian, M. Trost, M. G. Pucciarelli, G. Liu, P. Dehoux, L. Jansch, F. Garcia-del Portillo, O. Schneewind, P. Cossart, and T. E. L. G. Consortium. 2002b. Inactivation of the srtA gene in Listeria monocytogenes inhibits anchoring of surface proteins and affects virulence. Molec. Microbiol. 43:464–489.

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

Blackman, S. A., T. J. Smith, and S. J. Foster. 1998. The role of autolysins during vegetative growth of Bacillus subtilis. Microbiology 144:73–82.

Bockmann, R., C. Dickneite, B. Middendorf, W. Goebel, and Z. Sokolovic. 1996. Specific binding of the Listeria monocytogenes transcriptional regulator PrfA to target sequences requires additional factor(s) and is influenced by iron. Molec. Microbiol. 22:643–653.

Bohne, J., H. Kestler, C. Uebele, Z. Sokolovic, and W. Goebel. 1996. Differential regulation of the virulence genes of Listeria monocytogenes by the transcriptional activator PrfA. Molec. Microbiol. 20:1189–1198.

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

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

Bouwer, H. G., C. S. Nelson, B. L. Gibbins, D. A. Portnoy, and D. J. Hinrichs. 1992. Listeriolysin O is a target of the immune response to Listeria monocytogenes. J. Exp. Med. 175:1467–1471.

Bouwer, H. G., and D. J. Hinrichs. 1996. Cytotoxic-T-lymphocyte responses to epitopes of listeriolysin O and p60 following infection with Listeria monocytogenes. Infect. Immun. 64:2515–2522.

Boyartchuk, V. L., K. W. Broman, R. E. Mosher, S. E. D'Orazio, M. N. Starnbach, and W. F. Dietrich. 2001. Multigenic control of Listeria monocytogenes susceptibility in mice. Nat. Genet. 27:259–260.

Braun, L., S. Dramsi, P. Dehoux, H. Bierne, G. Lindahl, and P. Cossart. 1997. InlB: An invasion protein of Listeria monocytogenes with a novel type of surface association. Molec. Microbiol. 25:285–294.

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

Braun, L., F. Nato, B. Payrastre, J. C. Mazie, and P. Cossart. 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. Molec. Microbiol. 34:10–23.

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

Breitbach, K., K. Rottner, S. Klocke, M. Rohde, A. Jenzora, J. Wehland, and I. Steinmetz. 2003. Actin-based motility of Burkholderia pseudomallei involves the Arp 2/3 complex, but not N-WASP and Ena/VASP proteins. Cell. Microbiol. 5:385–393.

Brosch, R., M. Brett, B. Catimel, J. B. Luchansky, B. Ojeniyi, and J. Rocourt. 1996. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of Listeria monocytogenes via pulsed-field gel electrophoresis (PFGE). Int. J. Food Microbiol. 32:343–355.

Brouqui, P., M. L. Birg, and D. Raoult. 1994. Cytopathic effect, plaque formation, and lysis of Ehrlichia chaffeensis grown on continuous cell lines. Infect. Immun. 62:405–411.

Brundage, R. A., G. A. Smith, A. Camilli, J. A. Theriot, and D. A. Portnoy. 1993. Expression and phosphorylation of the Listeria monocytogenes ActA protein in mammalian cells. Proc. Natl. Acad. Sci. USA 90:11890–11894.

Brunt, L. M., D. A. Portnoy, and E. R. Unanue. 1990. Presentation of Listeria monocytogenes to CD8+ T cells requires secretion of hemolysin and intracellular bacterial growth. J. Immunol. 145:3540–3546.

Bubert, A., S. Kohler, and W. Goebel. 1992a. The homologous and heterologous regions within the iap gene allow genus- and species-specific identification of Listeria spp. by polymerase chain reaction. Appl. Environ. Microbiol. 58:2625–2632.

Bubert, A., M. Kuhn, W. Goebel, and S. Kohler. 1992b. Structural and functional properties of the p60 proteins from different Listeria species. J. Bacteriol. 174:8166–8171.

Bubert, A., P. Schubert, S. Kohler, R. Frank, and W. Goebel. 1994. Synthetic peptides derived from the Listeria monocytogenes p60 protein as antigens for the generation of polyclonal antibodies specific for secreted cell-free L. monocytogenes p60 proteins. Appl. Environ. Microbiol. 60:3120–3127.

Bubert, A., H. Kestler, M. Gotz, R. Bockmann, and W. Goebel. 1997. The Listeria monocytogenes iap gene as an indicator gene for the study of PrfA-dependent regulation. Molec. Gen. Genet. 256:54–62.

Bubert, A., Z. Sokolovic, S. K. Chun, L. Papatheodorou, A. Simm, and W. Goebel. 1999. Differential expression of Listeria monocytogenes virulence genes in mammalian host cells. Molec. Gen. Genet. 261:323–336.

Buchrieser, C., C. Rusniok, R. Kunst, P. Cossart, P. Glaser, and T. L. G. Consortium. 2003. Comparison of the genome sequences of Listeria monocytogenes and Listeria innocua: Clues for evolution and pathogenicity. FEMS Immunol. Med. Microbiol. 35:207–213.

Bula, C. J., J. Bille, and M. P. Glauser. 1995. An epidemic of food-borne listeriosis in western Switzerland: Description of 57 cases involving adults. Clin. Infect. Dis. 20:66–72.

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

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

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

Cameron, L. A., P. A. Giardini, F. S. Soo, and J. A. Theriot. 2000. Secrets of actin-based motility revealed by a bacterial pathogen. Nat. Rev. Molec. Cell. Biol. 1:110–119.

Camilli, A., D. A. Portnoy, and P. Yougman. 1990. Insertional mutagenesis of Listeria monocytogenes with a novel Tn917 derivative that allows direct cloning of DNA flanking transposon insertions. J. Bacteriol. 172:3738–3744.

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

Camilli, A., L. G. Tilney, and D. A. Portnoy. 1993. Dual roles of PlcA in Listeria monocytogenes pathogenesis. Molec. Microbiol. 8:143–157.

Carroll, S. A., T. Hain, U. Technow, A. Darji, P. Pashalidis, S. W. Joseph, and T. Chakraborty. 2003. Identification and characterization of a peptidoglycan hydrolase, MurA, of Listeria monocytogenes, a muramidase needed for cell separation. J. Bacteriol. 185: 6801–6808.

Chakraborty, T., M. Leimeister-Wachter, E. Domann, M. Hartl, W. Goebel, T. Nichterlein, and S. Notermans. 1992. Coordinate regulation of virulence genes in Listeria monocytogenes requires the product of the prfA gene. J. Bacteriol. 174:568–574.

Chamaillard, M., S. E. Girardin, J. Viala, and D. J. Philpott. 2003. Nods, Nalps and Naip: Intracellular regulators of bacterial-induced inflammation. Cell. Microbiol. 5:581–592.

Chambert, R., and M. F. Petit-Glatron. 1999. Anionic polymers of Bacillus subtilis cell wall modulate the folding rate of secreted proteins. FEMS Microbiol. Lett. 179:43–47.

Charpentier, E., G. Gerbaud, C. Jacquet, J. Rocourt, and P. Courvalin. 1995. Incidence of antibiotic resistance in Listeria species. J. Infect. Dis. 172:277–281.

Charpentier, E., and P. Courvalin. 1999. Antibiotic resistance in Listeria spp. Antimicrob. Agents Chemother. 43:2103–2108.

Cheers, C., and I. F. McKenzie. 1978. Resistance and susceptibility of mice to bacterial infection: Genetics of listeriosis. Infect. Immun. 19:755–762.

Chen, T., F. Grunert, A. Medina-Marino, and E. C. Gotschlich. 1997. Several carcinoembryonic antigens (CD66) serve as receptors for gonococcal opacity proteins. J. Exp. Med. 185:1557–1564.

Cheroutre-Vialette, M., I. Lebert, M. Hebraud, J. C. Labadie, and A. Lebert. 1998. Effects of pH or a(w) stress on growth of Listeria monocytogenes. Int. J. Food Microbiol. 42:71–77.

Chico-Calero, I., M. Suarez, B. Gonzalez-Zorn, M. Scortti, J. Slaghuis, W. Goebel, J. A. Vazquez-Boland, and T. E. L. G. Consortium. 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.

Cicchetti, G., P. Maurer, P. Wagener, and C. Kocks. 1999. Actin and phosphoinositide binding by the ActA protein of the bacterial pathogen Listeria monocytogenes. J. Biol. Chem. 274:33616–33626.

Cleveland, M. G., J. D. Gorham, T. L. Murphy, E. Tuomanen, and K. M. Murphy. 1996. Lipoteichoic acid preparations of Gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. Infect. Immun. 64:1906–1912.

Coconnier, M. H., M. Lorrot, A. Barbat, C. Laboisse, and A. L. Servin. 2000. Listeriolysin O-induced stimulation of mucin exocytosis in polarized intestinal mucin-secreting cells: Evidence for toxin recognition of membrane-associated lipids and subsequent toxin internalization through caveolae. Cell. Microbiol. 2:487–504.

Copp, J., M. Marino, M. Banerjee, P. Ghosh, and P. van der Geer. 2003. Multiple regions of internalin B contribute to its ability to turn on the Ras-mitogen-activated protein kinase pathway. J. Biol. Chem. 278: 7783–7789.

Cossart, P., and J. Mengaud. 1989a. Listeria monocytogenes: A model system for the molecular study of intracellular parasitism. Molec. Biol. Med. 6:463–474.

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

Cossart, P., and C. Kocks. 1994. The actin-based motility of the facultative intracellular pathogen Listeria monocytogenes. Molec. Microbiol. 13:395–402.

Cossart, P. 1997. Host/pathogen interactions: Subversion of the mammalian cell cytoskeleton by invasive bacteria. J. Clin. Invest. 99:2307–2311.

Cossart, P., and M. Lecuit. 1998. Interactions of Listeria monocytogenes with mammalian cells during entry and actin-based movement: Bacterial factors, cellular ligands and signaling. EMBO J. 17:3797–3806.

Cossart, P., and R. Jonquieres. 2000. Sortase, a universal target for therapeutic agents against gram-positive bacteria?. Proc. Natl. Acad. Sci. USA 97:5013–5015.

Cossart, P., and H. Bierne. 2001. The use of host cell machinery in the pathogenesis of Listeria monocytogenes. Curr. Opin. Immunol. 13:96–103.

Cossart, P., J. Pizarro-Cerda, and M. Lecuit. 2003. Invasion of mammalian cells by Listeria monocytogenes: Functional mimicry to subvert cellular functions. Trends Cell Biol. 13:23–31.

Cotter, P. D., N. Emerson, C. G. Gahan, and C. Hill. 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., C. G. Gahan, and C. Hill. 2000. Analysis of the role of the Listeria monocytogenes F0F1-ATPase operon in the acid tolerance response. Int. J. Food Microbiol. 60:137–146.

Cotter, P. D., C. G. Gahan, and C. Hill. 2001a. A glutamate decarboxylase system protects Listeria monocytogenes in gastric fluid. Molec. Microbiol. 40:465–475.

Cotter, P. D., K. O'Reilly, and C. Hill. 2001b. Role of the glutamate decarboxylase acid resistance system in the survival of Listeria monocytogenes LO28 in low pH foods. J. Food Prot. 64:1362–1368.

Cotter, P. D., C. M. Guinane, and C. Hill. 2002. The LisRK signal transduction system determines the sensitivity of Listeria monocytogenes to nisin and cephalosporins. Antimicrob. Agents Chemother. 46:2784–2790.

Cotter, P. D., and C. Hill. 2003. Surviving the acid test: Responses of Gram-positive bacteria to low pH. Microbiol. Molec. Biol. Rev. 67:429–453.

Cowart, R. E., and B. G. Foster. 1985. Differential effects of iron on the growth of Listeria monocytogenes: Minimum requirements and mechanism of acquisition. J. Infect. Dis. 151:721–730.

Cowart, R. E., J. Lashmet, M. E. McIntosh, and T. J. Adams. 1990. Adherence of a virulent strain of Listeria monocytogenes to the surface of a hepatocarcinoma cell line via lectin-substrate interaction. Arch. Microbiol. 153:282–286.

Cudmore, S., P. Cossart, G. Griffiths, and M. Way. 1995. Actin-based motility of vaccinia virus. Nature 378:636–638.

Czuprynski, C. J., N. G. Faith, and H. Steinberg. 2003. A/J mice are susceptible and C57BL/6 mice are resistant to Listeria monocytogenes infection by intragastric inoculation. Infect. Immun. 71:682–689.

Dabiri, G. A., J. M. Sanger, D. A. Portnoy, and F. S. Southwick. 1990. Listeria monocytogenes moves rapidly through the host-cell cytoplasm by inducing directional actin assembly. Proc. Natl. Acad. Sci. USA 87:6068–6072.

Dalgleish, A. G., P. C. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 312:763–767.

Dalton, C. B., C. C. Austin, J. Sobel, P. S. Hayes, W. F. Bibb, L. M. Graves, B. Swaminathan, M. E. Proctor, and P. M. Griffin. 1997. An outbreak of gastroenteritis and fever due to Listeria monocytogenes in milk. N. Engl. J. Med. 336:100–105.

Dancz, C. E., A. Haraga, D. A. Portnoy, and D. E. Higgins. 2002. Inducible control of virulence gene expression in Listeria monocytogenes: Temporal requirement of listeriolysin O during intracellular infection. J. Bacteriol. 184:5935–5945.

Darji, A., T. Chakraborty, J. Wehland, and S. Weiss. 1995. Listeriolysin generates a route for the presentation of exogenous antigens by major histocompatibility complex class I. Eur. J. Immunol. 25:2967–2971.

Darji, A., T. Chakraborty, J. Wehland, and S. Weiss. 1997. TAP-dependent major histocompatibility complex class I presentation of soluble proteins using listeriolysin. Eur. J. Immunol. 27:1353–1359.

David, V., E. Gouin, M. V. Troys, A. Grogan, A. W. Segal, C. Ampe, and P. Cossart. 1998. Identification of cofilin, coronin, Rac and capZ in actin tails using a Listeria affinity approach. J. Cell Sci. 111:2877–2884.

Decatur, A. L., and D. A. Portnoy. 2000. A PEST-like sequence in listeriolysin O essential for Listeria monocytogenes pathogenicity. Science 290:992–995.

De Chastellier, C., and P. Berche. 1994. Fate of Listeria monocytogenes in murine macrophages: Evidence for simultaneous killing and survival of intracellular bacteria. Infect. Immun. 62:543–553.

Delcour, J., T. Ferain, M. Deghorain, E. Palumbo, and P. Hols. 1999. The biosynthesis and functionality of the cell-wall of lactic acid bacteria. Ant. v. Leeuwenhoek 76:159–184.

Dhar, G., K. F. Faull, and O. Schneewind. 2000. Anchor structure of cell wall surface proteins in Listeria monocytogenes. Biochemistry 39:3725–3733.

Dietrich, G., A. Bubert, I. Gentschev, Z. Sokolovic, A. Simm, A. Catic, S. H. Kaufmann, J. Hess, A. A. Szalay, and W. Goebel. 1998. Delivery of antigen-encoding plasmid DNA into the cytosol of macrophages by attenuated suicide Listeria monocytogenes. Nat. Biotechnol. 16:181–185.

Dietrich, G., J. Hess, I. Gentschev, B. Knapp, S. H. Kaufmann, and W. Goebel. 2001. From evil to good: A cytolysin in vaccine development. Trends Microbiol. 9:23–28.

Domann, E., M. Leimeister-Wachter, W. Goebel, and T. Chakraborty. 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., J. Wehland, M. Rohde, S. Pistor, M. Hartl, W. Goebel, M. Leimeister-Wachter, M. Wuenscher, and T. Chakraborty. 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., S. Zechel, A. Lingnau, T. Hain, A. Darji, T. Nichterlein, J. Wehland, and T. Chakraborty. 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.

Dorig, R. E., A. Marcil, A. Chopra, and C. D. Richardson. 1993. The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell 75:295–305.

Doumith, M., C. Cazalet, N. Simoes, L. Frangeul, C. Jaquet, F. Kunst, P. Martin, P. Cossart, P. Glaser, and C. Buchrieser. 2004a. New aspects regarding evolution and virulence of Listeria monocytogenes revealed by comparative genomics. Infect. Immun. 72:1072–1083.

Dragic, T., V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton. 1996. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 381:667–673.

Dramsi, S., C. Kocks, C. Forestier, and P. Cossart. 1993. Internalin-mediated invasion of epithelial cells by List-

eria monocytogenes is regulated by the bacterial growth state, temperature and the pleiotropic activator prfA. Molec. Microbiol. 9:931–941.

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

Dramsi, S., P. Dehoux, M. Lebrun, P. L. Goossens, and P. Cossart. 1997. Identification of four new members of the internalin multigene family of Listeria monocytogenes EGD. Infect. Immun. 65:1615–1625.

Dramsi, S., S. Levi, A. Triller, and P. Cossart. 1998. Entry of Listeria monocytogenes into neurons occurs by cell-to-cell spread: An in vitro study. Infect. Immun. 66:4461–4468.

Dramsi, S., and P. Cossart. 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., F. Bourdichon, D. Cabanes, M. Lecuit, H. Fsihi, and P. Cossart. 2004. FbpA, a novel multifunctional Listeria monocytogenes virulence factor. Molec. Microbiol. 53(2):639–649.

Drevets, D. A., P. J. Leenen, and P. A. Campbell. 1993. Complement receptor type 3 (CD11b/CD18) involvement is essential for killing of Listeria monocytogenes by mouse macrophages. J. Immunol. 151:5431–5439.

Drevets, D. A., R. T. Sawyer, T. A. Potter, and P. A. Campbell. 1995. Listeria monocytogenes infects human endothelial cells by two distinct mechanisms. Infect. Immun. 63:4268–4276.

Drevets, D. A. 1998. Listeria monocytogenes virulence factors that stimulate endothelial cells. Infect. Immun. 66:232–238.

Dubail, I., P. Berche, and A. Charbit. 2000. Listeriolysin O as a reporter to identify constitutive and in vivo-inducible promoters in the pathogen Listeria monocytogenes. Infect. Immun. 68:3242–3250.

Dumont, J., and L. Cotoni. 1921. Bacille semblable à celui de rouget de porc rencontré dans le LCR d'un méningétique. Ann. Inst. Pasteur 5:625–633.

Dussurget, O., D. Cabanes, P. Dehoux, M. Lecuit, C. Buchrieser, P. Glaser, P. Cossart, and T. E. L. G. Consortium. 2002. Listeria monocytogenes bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. Molec. Microbiol. 45:1095–1106.

Dustoor, M., W. Croft, A. Fulton, and A. Blazkovec. 1977. Bacteriological and histopathological evaluation of guinea pigs after infection with Listeria monocytogenes. Infect. Immun. 15:916–924.

Edelson, B. T., P. Cossart, and E. R. Unanue. 1999. Cutting edge: Paradigm revisited—antibody provides resistance to Listeria infection. J. Immunol. 163:4087–4090.

Egile, C., T. P. Loisel, V. Laurent, R. Li, D. Pantaloni, P. J. Sansonetti, and M. F. Carlier. 1999. Activation of the CDC42 effector N-WASP by the Shigella flexneri IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. J. Cell. Biol. 146:1319–1332.

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

Engelbrecht, F., C. Dickneite, R. Lampidis, M. Gotz, U. DasGupta, and W. Goebel. 1998a. Sequence comparison of the chromosomal regions encompassing the internalin C genes (inlC) of Listeria monocytogenes and L. ivanovii. Molec. Gen. Genet. 257:186–197.

Engelbrecht, F., G. Dominguez-Bernal, J. Hess, C. Dickneite, L. Greiffenberg, R. Lampidis, D. Raffelsbauer, J. J. Daniels, J. Kreft, S. H. Kaufmann, J. A. Vazquez-Boland, and W. Goebel. 1998b. A novel PrfA-regulated chromosomal locus, which is specific for Listeria ivanovii, encodes two small, secreted internalins and contributes to virulence in mice. Molec. Microbiol. 30:405–417.

Farber, J. M., and P. I. Peterkin. 1991a. Listeria monocytogenes, a food-borne pathogen. Microbiol. Rev. 55:476–511.

Farber, J. M., E. Daley, F. Coates, N. Beausoleil, and J. Fournier. 1991b. Feeding trials of Listeria monocytogenes with a nonhuman primate model. J. Clin. Microbiol. 29:2606–2608.

Ferreira, A., C. P. O'Byrne, and K. J. Boor. 2001. Role of sigma(B) in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in Listeria monocytogenes. Appl. Environ. Microbiol. 67:4454–4457.

Ferreira, A., D. Sue, C. P. O'Byrne, and K. J. Boor. 2003. Role of Listeria monocytogenes sigma(B) in survival of lethal acidic conditions and in the acquired acid tolerance response. Appl. Environ. Microbiol. 69:2692–2698.

Fiedler, F., and G. J. Ruhland. 1987. Structure of Listeria monocytogenes cell walls. Bull. Inst. Pasteur 85:287–300.

Fiedler, F. 1988. Biochemistry of the cell surface of Listeria strains: A locating general view. Infection 16:92–97.

Finlay, B. B. 1999. Bacterial disease in diverse hosts. Cell 96:315–318.

Fischer, W., P. Rosel, and H. U. Koch. 1981. Effect of alanine ester substitution and other structural features of lipoteichoic acids on their inhibitory activity against autolysins of Staphylococcus aureus. J. Bacteriol. 146:467–475.

Fischer, W. 1988. Physiology of lipoteichoic acids in bacteria. Adv. Microb. Physiol. 29:233–302.

Fischer, W., T. Mannsfeld, and G. Hagen. 1990. On the basic structure of poly(glycerophosphate) lipoteichoic acids. Biochem. Cell. Biol. 68:33–43.

Flanary, P. L., R. D. Allen, L. Dons, and S. Kathariou. 1999. Insertional inactivation of the Listeria monocytogenes cheYA operon abolishes response to oxygen gradients and reduces the number of flagella. Can. J. Microbiol. 45:646–652.

Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 312:404–407.

Fortineau, N., P. Trieu-Cuot, O. Gaillot, E. Pellegrini, P. Berche, and J. L. Gaillard. 2000. Optimization of green fluorescent protein expression vectors for in vitro and in vivo detection of Listeria monocytogenes. Res. Microbiol. 151:353–360.

Francis, M. S., and C. J. Thomas. 1997. The Listeria monocytogenes gene ctpA encodes a putative P-type ATPase involved in copper-transport. Molec. Gen. Genet. 253:484–491.

Francis, K. P., D. Joh, C. Bellinger-Kawahara, M. J. Hawkinson, T. F. Purchio, and P. R. Contag. 2000. Monitoring bioluminescent Staphylococcus aureus infections in living mice using a novel luxABCDE construct. Infect. Immun. 68:3594–3600.

Fraser, K. R., D. Harvie, P. J. Coote, and C. P. O'Byrne. 2000. Identification and characterization of an ATP binding cassette L-carnitine transporter in Listeria monocytogenes. Appl. Environ. Microbiol. 66:4696–4704.

Frehel, C., M. A. Lety, N. Autret, J. L. Beretti, P. Berche, and A. Charbit. 2003. Capacity of ivanolysin O to replace listeriolysin O in phagosomal escape and in vivo survival of Listeria monocytogenes. Microbiology 149:611–620.

Freitag, N. E., P. Youngman, and D. A. Portnoy. 1992. Transcriptional activation of the Listeria monocytogenes hemolysin gene in Bacillus subtilis. J. Bacteriol. 174:1293–1298.

Freitag, N. E., L. Rong, and D. A. Portnoy. 1993. Regulation of the prfA transcriptional activator of Listeria monocytogenes: Multiple promoter elements contribute to intracellular growth and cell-to-cell spread. Infect. Immun. 61:2537–2544.

Freitag, N. E., and K. E. Jacobs. 1999. Examination of Listeria monocytogenes intracellular gene expression with the green fluorescent protein of Aequorea victoria. Infect. Immun. 67:1844–1852.

Freitag, N. E. 2000. Genetic tools for use with Listeria monocytogenes. *In:* V. A. Fischetti, R. P. Novick, J. J. Ferretti, D. A. Portnoy, and J. I. Rood (Eds.) Gram-positive Pathogens. ASM Press. Washington, DC. 488–498.

Friederich, E., E. Gouin, R. Hellio, C. Kocks, P. Cossart, and D. Louvard. 1995. Targeting of Listeria monocytogenes ActA protein to the plasma membrane as a tool to dissect both actin-based cell morphogenesis and ActA function. EMBO J. 14:27231–27234.

Frischknecht, F., and M. Way. 2001. Surfing pathogens and the lessons learned for actin polymerization. Trends Cell Biol. 11:30–38.

Gaeng, S., S. Scherer, H. Neve, and M. J. Loessner. 2000. Gene cloning and expression and secretion of Listeria monocytogenes bacteriophage-lytic enzymes in Lactococcus lactis. Appl. Environ. Microbiol. 66:2951–2958.

Gahan, C. G., J. O'Mahony, and C. Hill. 2001. Characterization of the groESL operon in Listeria monocytogenes: Utilization of two reporter systems (gfp and hly) for evaluating in vivo expression. Infect. Immun. 69:3924–3932.

Gaillard, J. L., P. Berche, and P. Sansonetti. 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., P. Berche, J. Mounier, S. Richard, and P. Sansonetti. 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., P. Berche, C. Frehel, E. Gouin, and P. Cossart. 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., F. Jaubert, and P. Berche. 1996. The inlAB locus mediates the entry of Listeria monocytogenes into hepatocytes in vivo. J. Exp. Med. 183:359–369.

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

Gandhi, A. J., B. Perussia, and H. Goldfine. 1993. Listeria monocytogenes phosphatidylinositol (PI)-specific phospholipase C has low activity on glycosyl-PI-anchored proteins. J. Bacteriol. 175:8014–8017.

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

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

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

Geginat, G., M. Lalic, M. Kretschmar, W. Goebel, H. Hof, D. Palm, and A. Bubert. 1998. Th1 cells specific for a secreted protein of Listeria monocytogenes are protective in vivo. J. Immunol. 160:6046–6055.

Geginat, G., T. Nichterlein, M. Kretschmar, S. Schenk, H. Hof, M. Lalic-Multhaler, W. Goebel, and A. Bubert. 1999. Enhancement of the Listeria monocytogenes p60-specific CD4 and CD8 T cell memory by nonpathogenic Listeria innocua. J. Immunol. 162:4781–4789.

Gellin, B. G., C. V. Broome, W. F. Bibb, R. E. Weaver, S. Gaventa, and L. Mascola. 1991. The epidemiology of listeriosis in the United States—1986: Listeriosis Study Group. Am. J. Epidemiol. 133:392–401.

Genovese, F., G. Mancuso, M. Cuzzola, C. Biondo, C. Beninati, D. Delfino, and G. Teti. 1999. Role of IL-10 in a neonatal mouse listeriosis model. J. Immunol. 163:2777–2782.

Geoffroy, C., J. L. Gaillard, J. E. Alouf, and P. Berche. 1987. Purification, characterization, and toxicity of the sulfhydryl-activated hemolysin listeriolysin O from Listeria monocytogenes. Infect. Immun. 55: 1641–1646.

Geoffroy, C., J. L. Gaillard, J. E. Alouf, and P. Berche. 1989. Production of thiol-dependent haemolysins by Listeria monocytogenes and related species. J. Gen. Microbiol. 135:481–487.

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

Ghebrehiwet, B., B. L. Lim, R. Kumar, X. Feng, and E. I. Peerschke. 2001. gC1q-R/p33, a member of a new class of multifunctional and multicompartmental cellular proteins, is involved in inflammation and infection. Immunol. Rev. 180:65–77.

Gholizadeh, Y., C. Poyart, M. Juvin, J. L. Beretti, J. Croize, P. Berche, and J. L. Gaillard. 1996. Serodiagnosis of listeriosis based upon detection of antibodies against recombinant truncated forms of listeriolysin O. J. Clin. Microbiol. 34:1391–1395.

Ghuysen, J. M., D. J. Tipper, and J. L. Strominger. 1966. Enzymes that degrade cell walls. Meth. Enzymol. 8:685–699.

Gilbert, R. J. 2002. Pore-forming toxins. Cell. Molec. Life Sci. 59:832–844.

Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok, A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couve, A. de Daruvar, P. Dehoux, E. Domann, G. Dominguez-Bernal, E. Duchaud, L. Durant, O. Dussurget, K. D. Entian, H. Fsihi, F. G. Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gomez-Lopez, T. Hain, J. Hauf, D. Jackson, L. M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurap-

kat, E. Madueno, A. Maitournam, J. M. Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. de Pablos, J. C. Perez-Diaz, R. Purcell, B. Remmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J. A. Vazquez-Boland, H. Voss, J. Wehland, and P. Cossart. 2001. Comparative genomics of Listeria species. Science 294:849–852.

Glomski, I. J., M. M. Gedde, A. W. Tsang, J. A. Swanson, and D. A. Portnoy. 2002. The Listeria monocytogenes hemolysin has an acidic pH optimum to compartmentalize activity and prevent damage to infected host cells. J. Cell Biol. 156:1029–1038.

Glomski, I. J., A. L. Decatur, and D. A. Portnoy. 2003. Listeria monocytogenes mutants that fail to compartmentalize listeriolysin O activity are cytotoxic, avirulent, and unable to evade host extracellular defenses. Infect. Immun. 71:6754–6765.

Goebel, W., J. Kreft, and R. Bockmann. 2000. Regulation of virulence genes in pathogenic Listeria spp. *In:* V. A. Fischetti, R. P. Novick, J. J. Ferretti, D. A. Portnoy, and J. I. Rood (Eds.) Gram-positive Pathogens. ASM Press. Washington, DC. 501–506.

Goetz, M., A. Bubert, G. Wang, I. Chico-Calero, J. A. Vazquez-Boland, M. Beck, J. Slaghuis, A. A. Szalay, and W. Goebel. 2001. Microinjection and growth of bacteria in the cytosol of mammalian host cells. Proc. Natl. Acad. Sci. USA 98:12221–12226.

Goldfine, H., and C. Knob. 1992. Purification and characterization of Listeria monocytogenes phosphatidylinositol-specific phospholipase C. Infect. Immun. 60:4059–4067.

Goldfine, H., N. C. Johnston, and C. Knob. 1993. Nonspecific phospholipase C of Listeria monocytogenes: Activity on phospholipids in Triton X-100-mixed micelles and in biological membranes. J. Bacteriol. 175:4298–4306.

Goldfine, H., S. J. Wadsworth, and N. C. Johnston. 2000. Activation of host phospholipases C and D in macrophages after infection with Listeria monocytogenes. Infect. Immun. 68:5735–5741.

Gonzalez-Zorn, B., G. Dominguez-Bernal, M. Suarez, M. T. Ripio, Y. Vega, S. Novella, and J. A. Vazquez-Boland. 1999. The smcL gene of Listeria ivanovii encodes a sphingomyelinase C that mediates bacterial escape from the phagocytic vacuole. Molec. Microbiol. 33:510–523.

Gouin, E., P. Dehoux, J. Mengaud, C. Kocks, and P. Cossart. 1995. iactA of Listeria ivanovii, although distantly related to Listeria monocytogenes actA, restores actin tail formation in an L. monocytogenes actA mutant. Infect. Immun. 63:2729–2737.

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

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

Goulet, V., and P. Marchetti. 1996. Listeriosis in 225 non-pregnant patients in 1992: Clinical aspects and outcome in relation to predisposing conditions. Scand. J. Infect. Dis. 28:367–374.

Goulet, V., J. Rocourt, I. Rebiere, C. Jacquet, C. Moyse, P. Dehaumont, G. Salvat, and P. Veit. 1998. Listeriosis outbreak associated with the consumption of rillettes in France in 1993. J. Infect. Dis. 177:155–160.

Gray-Owen, S. D., D. R. Lorenzen, A. Haude, T. F. Meyer, and C. Dehio. 1997. Differential Opa specificities for CD66 receptors influence tissue interactions and cellular response to Neisseria gonorrhea. Molec. Microbiol. 26:971–980.

Greenberg, J. W., W. Fischer, and K. A. Joiner. 1996. Influence of lipoteichoic acid structure on recognition by the macrophage scavenger receptor. Infect. Immun. 64:3318–3325.

Gregory, S. H., A. J. Sagnimeni, and E. J. Wing. 1996a. Bacteria in the bloodstream are trapped in the liver and killed by immigrating neutrophils. J. Immunol. 157:2514–2520.

Gregory, S. H., A. J. Sagnimeni, and E. J. Wing. 1996b. Expression of the inlAB operon by Listeria monocytogenes is not required for entry into hepatic cells in vivo. Infect. Immun. 64:3983–3986.

Gregory, S. H., A. J. Sagnimeni, and E. J. Wing. 1997. Internalin B promotes the replication of Listeria monocytogenes in mouse hepatocytes. Infect. Immun. 65:5137–5141.

Grenklo, S., M. Geese, U. Lindberg, J. Wehland, R. Karlsson, and A. S. Sechi. 2003. A crucial role for profilin-actin in the intracellular motility of Listeria monocytogenes. EMBO Rep. 4:523–529.

Grenningloh, R., A. Darji, J. Wehland, T. Chakraborty, and S. Weiss. 1997. Listeriolysin and IrpA are major protein targets of the human humoral response against Listeria monocytogenes. Infect. Immun. 65:3976–3980.

Grif, K., I. Hein, M. Wagner, E. Brandl, O. Mpamugo, J. McLauchlin, M. P. Dierich, and F. Allerberger. 2001. Listeria monocytogenes in the feces of healthy Austrians. Wien. Klin. Wochenschr. 113:737–742.

Grillot-Courvalin, C., S. Goussard, and P. Courvalin. 2002. Wild-type intracellular bacteria deliver DNA into mammalian cells. Cell. Microbiol. 4:177–186.

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

Gutekunst, K. A., B. P. Holloway, and G. M. Carlone. 1992a. DNA sequence heterogeneity in the gene encoding a 60-kilodalton extracellular protein of Listeria monocytogenes and other Listeria species. Can. J. Microbiol. 38:865–870.

Gutekunst, K. A., L. Pine, E. White, S. Kathariou, and G. M. Carlone. 1992b. A filamentous-like mutant of Listeria monocytogenes with reduced expression of a 60-kilodalton extracellular protein invades and grows in 3T6 and Caco-2 cells. Can. J. Microbiol. 38:843–851.

Guzman, C. A., M. Rohde, T. Chakraborty, E. Domann, M. Hudel, J. Wehland, and K. N. Timmis. 1995. Interaction of Listeria monocytogenes with mouse dendritic cells. Infect. Immun. 63:3665–3673.

Guzman, C. A., E. Domann, M. Rohde, D. Bruder, A. Darji, S. Weiss, J. Wehland, T. Chakraborty, and K. N. Timmis. 1996. Apoptosis of mouse dendritic cells is triggered by listeriolysin, the major virulence determinant of Listeria monocytogenes. Molec. Microbiol. 20:119–126.

Haas, A., M. Dumbsky, and J. Kreft. 1992. Listeriolysin genes: Complete sequence of ilo from Listeria ivanovii and of lso from Listeria seeligeri. Biochim. Biophys. Acta 1130:81–84.

Haas, C. N., and A. Thayyar-Madabusi. 1999. Development and validation of dose-response relationship for Listeria monocytogenes. Quant. Microbiol. 1:89–102.

Hanawa, T., T. Yamamoto, and S. Kamiya. 1995. Listeria monocytogenes can grow in macrophages without the

aid of proteins induced by environmental stresses. Infect. Immun. 63:4595–4599.

Hanawa, T., M. Fukuda, H. Kawakami, H. Hirano, S. Kamiya, and T. Yamamoto. 1999. The Listeria monocytogenes DnaK chaperone is required for stress tolerance and efficient phagocytosis with macrophages. Cell Stress Chaperones 4:118–128.

Haraga, A., and S. I. Miller. 2003. A Salmonella enterica serovar typhimurium translocated leucine-rich repeat effector protein inhibits NF-kappa B-dependent gene expression. Infect. Immun. 71:4052–4058.

Hardy, J., K. Francis, K. Gibbs, and C. Contag. 2002. *In:* Presented at the Integrative Approaches in Microbial Pathogenesis, November 2002. Paris, France. 13–16.

Harrington-Fowler, L., P. M. Henson, and M. S. Wilder. 1981. Fate of Listeria monocytogenes in resident and activated macrophages. Infect. Immun. 33:11–16.

Hartman, A. B., M. Venkatesan, E. V. Oaks, and J. M. Buysse. 1990. Sequence and molecular characterization of a multicopy invasion plasmid antigen gene, ipaH, of Shigella flexneri. J. Bacteriol. 172:1905–1915.

Harty, J. T., and E. G. Pamer. 1995. CD8 T lymphocytes specific for the secreted p60 antigen protect against Listeria monocytogenes infection. J. Immunol. 9:4642–4650.

Harvill, E. T., and J. F. Miller. 2000. Manipulating the host to study bacterial virulence. Curr. Opin. Microbiol. 3:93–96.

Hauf, N., W. Goebel, F. Fiedler, Z. Sokolovic, and M. Kuhn. 1997. Listeria monocytogenes infection of P388D1 macrophages results in a biphasic NF-kappaB (RelA/p50) activation induced by lipoteichoic acid and bacterial phospholipases and mediated by IkappaBalpha and IkappaBbeta degradation. Proc. Natl. Acad. Sci. USA 94:9394–9399.

Hauf, N., W. Goebel, F. Fiedler, and M. Kuhn. 1999. Listeria monocytogenes infection of Caco-2 human epithelial cells induces activation of transcription factor NF-kappa B/Rel-like DNA binding activities. FEMS Microbiol. Lett. 178:117–122.

Havell, E. A. 1986. Synthesis and secretion of interferon by murine fibroblasts in response to intracellular Listeria monocytogenes. Infect. Immun. 54:787–792.

Hebraud, M., and J. Guzzo. 2000. The main cold shock protein of Listeria monocytogenes belongs to the family of ferritin-like proteins. FEMS Microbiol. Lett. 190:29–34.

Heilmann, C., M. Hussain, G. Peters, and F. Gotz. 1997. Evidence for autolysin-mediated primary attachment of Staphylococcus epidermidis to a polystyrene surface. Molec. Microbiol. 24:1013–1024.

Hell, W., H. G. Meyer, and S. G. Gatermann. 1998. Cloning of aas, a gene encoding a Staphylococcus saprophyticus surface protein with adhesive and autolytic properties. Molec. Microbiol. 29:871–881.

Herd, M., and C. H. Kocks. 2001. Gene fragments distinguishing an epidemic-associated strain from a virulent prototype strain of Listeria monocytogenes belong to a distinct functional subset of genes and partially cross hybridize with other Listeria species. Infect. Immun. 69:3972–3979.

Herler, M., A. Bubert, M. Goetz, Y. Vega, J. A. Vazquez-Boland, and W. Goebel. 2001. Positive selection of mutations leading to loss or reduction of transcriptional activity of PrfA, the central regulator of Listeria monocytogenes virulence. J. Bacteriol. 183:5562–5570.

Hess, J., I. Gentschev, G. Szalay, C. Ladel, A. Bubert, W. Goebel, and S. H. Kaufmann. 1995. Listeria monocytogenes p60 supports host cell invasion by and in vivo survival of attenuated Salmonella typhimurium. Infect. Immun. 63:2047–2053.

Hether, N. W., and L. L. Jackson. 1983a. Lipoteichoic acid from Listeria monocytogenes. J. Bacteriol. 156:809–817.

Hether, N. W., P. A. Campbell, L. A. Baker, and L. L. Jackson. 1983b. Chemical composition and biological functions of Listeria monocytogenes cell wall preparations. Infect. Immun. 39:1114–1121.

Hill, C., P. D. Cotter, R. D. Sleator, and C. G. M. Gahan. 2002. Bacterial stress response in Listeria monocytogenes: Jumping the hurdles imposed by minimal processing. Int. Dairy J. 12:273–283.

Hiltbold, E. M., S. A. Safley, and H. K. Ziegler. 1996. The presentation of class I and class II epitopes of listeriolysin O is regulated by intracellular localization and by intercellular spread of Listeria monocytogenes. J. Immunol. 157:1163–1175.

Hodgson, D. A. 2000. Generalized transduction of serotype 1/2 and serotype 4b strains of Listeria monocytogenes. Molec. Microbiol. 35:312–323.

Hof, H., and P. Hefner. 1988. Pathogenicity of Listeria monocytogenes in comparison to other Listeria species. Infection 16:S141–S144.

Hof, H., T. Nichterlein, and M. Kretschmar. 1997. Management of listeriosis. Clin. Microbiol. Rev. 10:345–357.

Hooper, L. V., and J. I. Gordon. 2001. Commensal host-bacterial relationships in the gut. Science 292:1115–1118.

Hugues, A. H., I. C. Hancock, and J. Baddiley. 1973. The function of teichoic acids in cation control in bacterial membranes. Biochem. J. 132:83–93.

Huillet, E., S. Larpin, P. Pardon, and P. Berche. 1999. Identification of a new locus in Listeria monocytogenes involved in cellobiose-dependent repression of hly expression. FEMS Microbiol. Lett. 174:265.

Huleatt, J. W., I. Pilip, K. Kerksiek, and E. G. Pamer. 2001. Intestinal and splenic T cell responses to enteric Listeria monocytogenes infection: Distinct repertoires of responding CD8 T lymphocytes. J. Immunol. 166:4065–4073.

Hyyrylainen, H. L., M. Vitikainen, J. Thwaite, H. Wu, M. Sarvas, C. R. Harwood, V. P. Kontinen, and K. Stephenson. 2000. D-Alanine substitution of teichoic acids as a modulator of protein folding and stability at the cytoplasmic membrane/cell wall interface of Bacillus subtilis. J. Biol. Chem. 275:26696–26703.

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

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

Ito, Y., I. Kawamura, C. Kohda, H. Baba, T. Nomura, T. Kimoto, I. Watanabe, and M. Mitsuyama. 2003. Seeligeriolysin O, a cholesterol-dependent cytolysin of Listeria seeligeri, induces gamma interferon from spleen cells of mice. Infect. Immun. 71:234–241.

Ivanov, I. 1962. Untersuchungen über die Listeriose der Schaffe in Bulgarien. Monatschr. Vet. Med. 17:729–736.

Jacobs, T., A. Darji, N. Frahm, M. Rohde, J. Wehland, T. Chakraborty, and S. Weiss. 1998. Listeriolysin O: Cholesterol inhibits cytolysis but not binding to cellular membranes. Molec. Microbiol. 28:1081–1089.

Jacquet, C., M. Doumith, J. I. Gordon, P. M. V. Martin, and P. Cossart. 2004. A molecular marker for evaluating the

pathogenic potential of foodborne Listeria monocytogenes. J. Infect. Dis. 189(11):2094–2100.

Jaradat, Z. W., J. W. Wampler, and A. W. Bhunia. 2003. A Listeria adhesion protein-deficient Listeria monocytogenes strain shows reduced adhesion primarily to intestinal cell lines. Med. Microbiol. Immunol. (Berlin) 192:85–91.

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

Jolliffe, L. K., R. J. Doyle, and U. N. Streips. 1981. The energized membrane and cellular autolysis in Bacillus subtilis. Cell 25:753–763.

Jones, S., K. Preiter, and D. A. Portnoy. 1996. Conversion of an extracellular cytolysin into a phagosome-specific lysin which supports the growth of an intracellular pathogen. Molec. Microbiol. 21:1219–1225.

Jonquieres, R., H. Bierne, J. Mengaud, and P. Cossart. 1998. The inlA gene of Listeria monocytogenes LO28 harbors a nonsense mutation resulting in release of internalin. Infect. Immun. 66:3420–3422.

Jonquieres, R., H. Bierne, F. Fiedler, P. Gounon, and P. Cossart. 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. Molec. Microbiol. 34:902–914.

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

Jurado, R. L., M. M. Farley, E. Pereira, R. C. Harvey, A. Schuchat, J. D. Wenger, and D. S. Stephens. 1993. Increased risk of meningitis and bacteremia due to Listeria monocytogenes in patients with human immunodeficiency virus infection. Clin. Infect. Dis. 17:224–227.

Kallipolitis, B. H., and H. Ingmer. 2001. Listeria monocytogenes response regulators important for stress tolerance and pathogenesis. FEMS Microbiol. Lett. 204: 111–115.

Kallipolitis, B. H., H. Ingmer, C. G. Gahan, C. Hill, and L. Sogaard-Andersen. 2003. CesRK, a two-component signal transduction system in Listeria monocytogenes, responds to the presence of cell wall-acting antibiotics and affects beta-lactam resistance. Antimicrob. Agents Chemother. 47:3421–3429.

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

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

Kaufmann, S. H. 1988. Listeria monocytogenes specific T-cell lines and clones. Infection 16:S128–S136.

Kautter, D. A., S. J. Silverman, G., R. W., and F., D. J. 1963. Virulence of Listeria monocytogenes for experimental animals. J. Infect. Dis. 112:167–180.

Kayal, S., A. Lilienbaum, C. Poyart, S. Memet, A. Israel, and P. Berche. 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. Molec. Microbiol. 31:1709–1722.

Kayal, S., A. Lilienbaum, O. Join-Lambert, X. Li, A. Israel, and P. Berche. 2002. Listeriolysin O secreted by Listeria monocytogenes induces NF-kappaB signalling by activating the IkappaB kinase complex. Molec. Microbiol. 44:1407–1419.

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

Keller, R., W. Fischer, R. Keist, and S. Bassetti. 1992. Macrophage response to bacteria: Induction of marked secretory and cellular activities by lipoteichoic acids. Infect. Immun. 60:3664–3672.

Kemper, M. A., M. M. Urrutia, T. J. Beveridge, A. L. Koch, and R. J. Doyle. 1993. Proton motive force may regulate cell wall-associated enzymes of Bacillus subtilis. J. Bacteriol. 175:5690–5696.

Khan, M. A., A. Seaman, and M. Woodbine. 1972. Proceedings: The pathogenicity of Listeria monocytogenes. Acta Microbiol. Acad. Sci. Hung. 19:421–426.

Kimoto, T., I. Kawamura, C. Kohda, T. Nomura, K. Tsuchiya, Y. Ito, I. Watanabe, T. Kaku, E. Setianingrum, and M. Mitsuyama. 2003. Differences in gamma interferon production induced by listeriolysin O and ivanolysin O result in different levels of protective immunity in mice infected with Listeria monocytogenes and Listeria ivanovii. Infect. Immun. 71:2447–2454.

Klarsfeld, A., P. L. Goossens, and P. Cossart. 1994. Five Listeria monocytogenes genes preferentially expressed in infected mammalian cells: plcA, purH, purD, pyrE and an arginine ABC transporter gene, arpJ. Molec. Microbiol. 13:585–597.

Ko, R., L. T. Smith, and G. M. Smith. 1994. Glycine betaine confers enhanced osmotolerance and cryotolerance on Listeria monocytogenes. J. Bacteriol. 176:426–431.

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

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

Kocks, C., R. Hellio, P. Gounon, H. Ohayon, and P. Cossart. 1993. Polarized distribution of Listeria monocytogenes surface protein ActA at the site of directional actin assembly. J. Cell. Sci. 105:699–710.

Kocks, C., J. B. Marchand, E. Gouin, H. d'Hauteville, P. J. Sansonetti, M. F. Carlier, and P. Cossart. 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. Molec. Microbiol. 18:413–423.

Kohda, C., I. Kawamura, H. Baba, T. Nomura, Y. Ito, T. Kimoto, I. Watanabe, and M. Mitsuyama. 2002. Dissociated linkage of cytokine-inducing activity and cytotoxicity to different domains of listeriolysin O from Listeria monocytogenes. Infect. Immun. 70:1334–1341.

Kohler, S., A. Bubert, M. Vogel, and W. Goebel. 1991. Expression of the iap gene coding for protein p60 of Listeria monocytogenes is controlled on the posttranscriptional level. J. Bacteriol. 173:4668–4674.

Kolb-Maurer, A., S. Pilgrim, E. Kampgen, A. D. McLellan, E. B. Brocker, W. Goebel, and I. Gentschev. 2001. Antibodies against listerial protein 60 act as an opsonin for

phagocytosis of Listeria monocytogenes by human dendritic cells. Infect. Immun. 69:3100–3109.

Kolb-Maurer, A., U. Kammerer, M. Maurer, I. Gentschev, E. B. Brocker, P. Rieckmann, and E. Kampgen. 2003. Production of IL-12 and IL-18 in human dendritic cells upon infection by Listeria monocytogenes. FEMS Immunol. Med. Microbiol. 35:255–262.

Kolstad, J., L. M. Rorvik, and P. E. Granum. 1991. Characterization of plasmids from Listeria sp. Int. J. Food Microbiol. 12:123–131.

Kreft, J., and J. A. Vazquez-Boland. 2001. Regulation of virulence genes in Listeria. Int. J. Med. Microbiol. 291:145–157.

Krull, M., R. Nost, S. Hippenstiel, E. Domann, T. Chakraborty, and N. Suttorp. 1997. Listeria monocytogenes potently induces up-regulation of endothelial adhesion molecules and neutrophil adhesion to cultured human endothelial cells. J. Immunol. 159:1970–1976.

Kuhn, M., S. Kathariou, and W. Goebel. 1988. Hemolysin supports survival but not entry of the intracellular bacterium Listeria monocytogenes. Infect. Immun. 56:79–82.

Kuhn, M., and W. Goebel. 1989. Identification of an extracellular protein of Listeria monocytogenes possibly involved in intracellular uptake by mammalian cells. Infect. Immun. 57:55–61.

Kussel-Andermann, P., A. El-Amraoui, S. Safieddine, S. Nouaille, I. Perfettini, M. Lecuit, P. Cossart, U. Wolfrum, and C. Petit. 2000. Vezatin, a novel transmembrane protein, bridges myosin VIIA to the cadherin-catenins complex. EMBO J. 15:6020–6029.

Labischinski, H., D. Naumann, and W. Fischer. 1991. Small and medium-angle X-ray analysis of bacterial lipoteichoic acid phase structure. Eur. J. Biochem. 202:1269–1274.

Lampidis, R., R. Gross, Z. Sokolovic, W. Goebel, and J. Kreft. 1994. The virulence regulator protein of Listeria ivanovii is highly homologous to PrfA from Listeria monocytogenes and both belong to the Crp-Fnr family of transcription regulators. Molec. Microbiol. 13:141–151.

Lara-Tejero, M., and E. G. Pamer. 2004. T cell responses to Listeria monocytogenes. Curr. Opin. Microbiol. 7:45–50.

Lasa, I., V. David, E. Gouin, J. B. Marchand, and P. Cossart. 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. Molec. Microbiol. 18:425–436.

Lasa, I., E. Gouin, M. Goethals, K. Vancompernolle, V. David, J. Vandekerckhove, and P. Cossart. 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.

Lauer, P., M. Y. Chow, M. J. Loessner, D. A. Portnoy, and R. Calendar. 2002. Construction, characterization, and use of two Listeria monocytogenes site-specific phage integration vectors. J. Bacteriol. 184:4177–4186.

Lebrun, M., J. Loulergue, E. Chaslus-Dancla, and A. Audurier. 1992. Plasmids in Listeria monocytogenes in relation to cadmium resistance. Appl. Environ. Microbiol. 58:3183–3186.

Lebrun, M., A. Audurier, and P. Cossart. 1994. Plasmid-borne cadmium resistance genes in Listeria monocytogenes are present on Tn5422, a novel transposon closely related to Tn917. J. Bacteriol. 176:3049–3061.

Lebrun, M., J. Mengaud, H. Ohayon, F. Nato, and P. Cossart. 1996. Internalin must be on the bacterial surface to mediate entry of Listeria monocytogenes into epithelial cells. Molec. Microbiol. 21:579–592.

Lecuit, M., H. Ohayon, L. Braun, J. Mengaud, and P. Cossart. 1997. Internalin of Listeria monocytogenes with an intact leucine-rich repeat region is sufficient to promote internalization. Infect. Immun. 65:5309–5319.

Lecuit, M., S. Dramsi, C. Gottardi, M. Fedor-Chaiken, B. Gumbiner, and P. Cossart. 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., R. Hurme, J. Pizarro-Cerda, H. Ohayon, B. Geiger, and P. Cossart. 2000. A role for alpha-and beta-catenins in bacterial uptake. Proc. Natl. Acad. Sci. USA 97:10008–10013.

Lecuit, M., and C. Cossart. 2001a. Listeria monocytogenes. *In:* S. Sussman (Ed.) Molecular Medical Microbiology. Academic Press. London, UK. 2:1437–1462.

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

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

Lecuit, M. 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, K. D., Y. K. Oh, D. A. Portnoy, and J. A. Swanson. 1996. Delivery of macromolecules into cytosol using liposomes containing hemolysin from Listeria monocytogenes. J. Biol. Chem. 271:7249–7252.

Leimeister-Wachter, M., and T. Chakraborty. 1989. Detection of listeriolysin, the thiol-dependent hemolysin in Listeria monocytogenes, Listeria ivanovii, and Listeria seeligeri. Infect. Immun. 57:2350–2357.

Leimeister-Wachter, M., C. Haffner, E. Domann, W. Goebel, and T. Chakraborty. 1990. Identification of a gene that positively regulates expression of listeriolysin, the major virulence factor of Listeria monocytogenes. Proc. Natl. Acad. Sci. USA 87:8336–8340.

Leimeister-Wachter, M., E. Domann, and T. Chakraborty. 1991. Detection of a gene encoding a phosphatidylinositol-specific phospholipase C that is coordinately expressed with listeriolysin in Listeria monocytogenes. Molec. Microbiol. 5:361–366.

Leimeister-Wachter, M., E. Domann, and T. Chakraborty. 1992. The expression of virulence genes in Listeria monocytogenes is thermoregulated. J. Bacteriol. 174:947–952.

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

Lety, M. A., C. Frehel, I. Dubail, J. L. Beretti, S. Kayal, P. Berche, and A. Charbit. 2001. Identification of a PEST-like motif in listeriolysin O required for phagosomal escape and for virulence in Listeria monocytogenes. Molec. Microbiol. 39:1124–1139.

Lety, M. A., C. Frehel, P. Berche, and A. Charbit. 2002. Critical role of the N-terminal residues of listeriolysin O in phagosomal escape and virulence of Listeria monocytogenes. Molec. Microbiol. 46:367–379.

Lety, M. A., C. Frehel, J. L. Beretti, P. Berche, and A. Charbit. 2003. Modification of the signal sequence cleavage site

of listeriolysin O does not affect protein secretion but impairs the virulence of Listeria monocytogenes. Microbiology 149:1249–1255.

Leung, K. Y., and S. C. Straley. 1989. The yopM gene of Yersinia pestis encodes a released protein having homology with the human platelet surface protein GPIb alpha. J. Bacteriol. 171:4623–4632.

Lievin-Le Moal, V., G. Huet, J. P. Aubert, J. Bara, M. E. Forgue-Lafitte, A. L. Servin, and M. H. Coconnier. 2002. Activation of mucin exocytosis and upregulation of MUC genes in polarized human intestinal mucin-secreting cells by the thiol-activated exotoxin listeriolysin O. Cell. Microbiol. 4:515–529.

Lingnau, A., E. Domann, M. Hudel, M. Bock, T. Nichterlein, J. Wehland, and T. Chakraborty. 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.

Lingnau, A., T. Chakraborty, K. Niebuhr, E. Domann, and J. Wehland. 1996. Identification and purification of novel internalin-related proteins in Listeria monocytogenes and Listeria ivanovii. Infect. Immun. 64:1002–1006.

Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, and R. Weaver. 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. 319:823–828.

Loessner, M. J., and S. Scherer. 1995a. Organization and transcriptional analysis of the Listeria phage A511 late gene region comprising the major capsid and tail sheath protein genes cps and tsh. J. Bacteriol. 177:6601–6609.

Loessner, M. J., G. Wendlinger, and S. Scherer. 1995b. Heterogeneous endolysins in Listeria monocytogenes bacteriophages: A new class of enzymes and evidence for conserved holin genes within the siphoviral lysis cassettes. Molec. Microbiol. 16:1231–1241.

Loessner, M. J., R. B. Inman, P. Lauer, and R. Calendar. 2000. Complete nucleotide sequence, molecular analysis and genome structure of bacteriophage A118 of Listeria monocytogenes: Implications for phage evolution. Molec. Microbiol. 2:324–340.

Loessner, M. J., K. Kramer, F. Ebel, and S. Scherer. 2002. C-terminal domains of Listeria monocytogenes bacteriophage murein hydrolases determine specific recognition and high-affinity binding to bacterial cell wall carbohydrates. Molec. Microbiol. 44:335–349.

Loisel, T. P., R. Boujemaa, D. Pantaloni, and M. F. Carlier. 1999. Reconstitution of actin-based motility of Listeria and Shigella using pure proteins. Nature 401:613–616.

Lorber, B. 1997. Listeriosis. Clin. Infect. Dis. 24:1–9.

Louria, D. B., T. Hensle, D. Armstrong, H. S. Collins, A. Blevins, D. Krugman, and M. Buse. 1967. Listeriosis complicating malignant disease: A new association. Ann. Intern. Med. 67:260–281.

Low, J. C., and C. P. Renton. 1985. Septicaemia, encephalitis and abortions in a housed flock of sheep caused by Listeria monocytogenes type 1/2. Vet. Rec. 116:147–150.

Luchansky, J. B., P. M. Muriana, and T. R. Klaenhammer. 1988. Application of electroporation for transfer of plasmid DNA to Lactobacillus, Lactococcus, Leuconostoc, Listeria, Pediococcus, Bacillus, Staphylococcus, Enterococcus and Propionibacterium. Molec. Microbiol. 2:637–646.

Luft, B. J., and J. S. Remington. 1982. Effect of pregnancy on resistance to Listeria monocytogenes and Toxoplasma gondii infections in mice. Infect. Immun. 38:1164–1171.

MacDonald, T. T., and P. B. Carter. 1980. Cell-mediated immunity to intestinal infection. Infect. Immun. 28:516–523.

Machesky, L. M. 1997. Cell motility: Complex dynamics at the leading edge. Curr. Biol. 7:R164–R167.

Machesky, L. M., and R. H. Insall. 1998. Scar1 and the related Wiskott-Aldrich Syndrome Protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. Curr. Biol. 8:1347–1356.

Machner, M. P., S. Frese, W. D. Schubert, V. Orian-Rousseau, E. Gherardi, J. Wehland, H. H. Niemann, and D. W. Heinz. 2003. Aromatic amino acids at the surface of InlB are essential for host cell invasion by Listeria monocytogenes. Molec. Microbiol. 48:1525–1536.

Mackaness, G. B. 1962. Cellular resistance to infection. J. Exp. Med. 118:381–406.

Mackaness, G. B., and W. C. Hill. 1969. The effect of antilymphocyte globulin on cell-mediated resistance to infection. J. Exp. Med. 129:993–1012.

Makhov, A. M., J. H. Hannah, M. J. Brennan, B. L. Trus, E. Kocsis, J. F. Conway, P. T. Wingfield, M. N. Simon, and A. C. Steven. 1994. Filamentous hemagglutinin of Bordetella pertussis: A bacterial adhesin formed as a 50-nm monomeric rigid rod based on a 19-residue repeat motif rich in beta strands and turns. J. Molec. Biol. 241:110–124.

Manohar, M., D. O. Baumann, N. A. Bos, and J. J. Cebra. 2001. Gut colonization of mice with ActA-negative mutant of Listeria monocytogenes can stimulate a humoral mucosal immune response. Infect. Immun. 69:3542–3549.

Mansell, A., L. Braun, P. Cossart, and L. A. O'Neill. 2000. A novel function of InlB from Listeria monocytogenes activation by NF-KB in J774 macrophages. Cell. Microbiol. 2:127–136.

Mansell, A., N. Khelef, P. Cossart, and L. A. O'Neill. 2001. Internalin B activates nuclear factor-kB via Ras, phosphoinositide 3-kinase and Akt. J. Biol. Chem. 276:43597–43603.

Marco, A. J., J. Altimira, N. Prats, S. Lopez, L. Dominguez, M. Domingo, and V. Briones. 1997. Penetration of Listeria monocytogenes in mice infected by the oral route. Microb. Pathogen. 23:255–263.

Marino, M., L. Braun, P. Cossart, and P. Ghosh. 1999. Structure of the lnlB leucine-rich repeats, a domain that triggers host cell invasion by the bacterial pathogen L. monocytogenes. Molec. Cell. 4:1063–1072.

Marino, M., M. Banerjee, R. Jonquieres, P. Cossart, and P. Ghosh. 2002. GW domains of the Listeria monocytogenes invasion protein InlB are SH3-like and mediate binding to host ligands. EMBO J. 21:5623–5634.

Marquis, H., H. G. Bouwer, D. J. Hinrichs, and D. A. Portnoy. 1993. Intracytoplasmic growth and virulence of Listeria monocytogenes auxotrophic mutants. Infect. Immun. 61:3756–3760.

Marquis, H., V. Doshi, and D. A. Portnoy. 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.

Marquis, H., H. Goldfine, and D. A. Portnoy. 1997. Proteolytic pathways of activation and degradation of a bacterial phospholipase C during intracellular infection by Listeria monocytogenes. J. Cell. Biol. 137:1381–1392.

Marquis, H., and E. J. Hager. 2000. pH-regulated activation and release of a bacteria-associated phospholipase C

during intracellular infection by Listeria monocytogenes. Molec. Microbiol. 35:289–298.

Marx, J. 2003. Cell biology: How cells step out. Science 302:214–216.

Mathew, E., G. E. Hardee, C. F. Bennett, and K. D. Lee. 2003. Cytosolic delivery of antisense oligonucleotides by listeriolysin O-containing liposomes. Gene Ther. 10:1105–1115.

Mazmanian, S. K., G. Liu, H. Ton-That, and O. Schneewind. 1999. Staphylococcus aureus sortase, an enzyme that anchors surface proteins to the cell wall. Science 285:760–763.

Mazmanian, S. K., H. Ton-That, and O. Schneewind. 2001. Sortase-catalysed anchoring of surface proteins to the cell wall of Staphylococcus aureus. Molec. Microbiol. 40:1049–1057.

Mazmanian, S. K., H. Ton-That, K. Su, and O. Schneewind. 2002. An iron-regulated sortase anchors a class of surface protein during Staphylococcus aureus pathogenesis. Proc. Natl. Acad. Sci. USA 99:2293–2298.

McClure, P. J., T. M. Kelly, and T. A. Roberts. 1991. The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of Listeria monocytogenes. Int. J. Food Microbiol. 14:77–91.

McDonald, C., P. O. Vacratsis, J. B. Bliska, and J. E. Dixon. 2003. The Yersinia virulence factor YopM forms a novel protein complex with two cellular kinases. J. Biol. Chem. 278:18514–18523.

McLauchlin, J., A. Audurier, A. Frommelt, P. Gerner-Smidt, C. Jacquet, M. J. Loessner, N. van der Mee-Marquet, J. Rocourt, S. Shah, and D. Wilhelms. 1996. WHO study on subtyping Listeria monocytogenes: Results of phage-typing. Int. J. Food Microbiol. 32:289–299.

McLaughlan, A. M., and S. J. Foster. 1997. Characterisation of the peptidoglycan hydrolases of Listeria monocytogenes EGD. FEMS Microbiol. Lett. 152:149–154.

Mencikova, E. 1989. Phospholipase C in Listeria. Acta Microbiol. Hung. 36:321–325.

Mendelsohn, C. L., E. Wimmer, and V. R. Racaniello. 1989. Cellular receptor for poliovirus: Molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell 56: 855–865.

Mengaud, J., J. Chenevert, C. Geoffroy, J. L. Gaillard, and P. Cossart. 1987. Identification of the structural gene encoding the SH-activated hemolysin of Listeria monocytogenes: Listeriolysin O is homologous to streptolysin O and pneumolysin. Infect. Immun. 55: 3225–3227.

Mengaud, J., C. Braun-Breton, and P. Cossart. 1991a. Identification of phosphatidylinositol-specific phospholipase C activity in Listeria monocytogenes: A novel type of virulence factor?. Molec. Microbiol. 5:367–372.

Mengaud, J., S. Dramsi, E. Gouin, J. A. Vazquez-Boland, G. Milon, and P. Cossart. 1991b. Pleiotropic control of Listeria monocytogenes virulence factors by a gene that is autoregulated. Molec. Microbiol. 5:2273–2283.

Mengaud, J. C. G., and P. Cossart. 1991c. Identification of a new operon involved in Listeria monocytogenes virulence: Its first gene encodes a protein homologous to bacterial metalloproteases. Infect. Immun. 59:1043–1049.

Mengaud, J., H. Ohayon, P. Gounon, R. M. Mege, and P. Cossart. 1996. E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells. Cell 84:923–932.

Merchante, R., H. M. Pooley, and D. Karamata. 1995. A periplasm in Bacillus subtilis. J. Bacteriol. 177:6176–6183.

Miao, E. A., M. Brittnacher, A. Haraga, R. L. Jeng, M. D. Welch, and S. I. Miller. 2003. Salmonella effectors translocated across the vacuolar membrane interact with the actin cytoskeleton. Molec. Microbiol. 48:401–415.

Michel, E., K. A. Reich, R. Favier, P. Berche, and P. Cossart. 1990. Attenuated mutants of the intracellular bacterium Listeria monocytogenes obtained by single amino acid substitutions in listeriolysin O. Molec. Microbiol. 4:2167–2178.

Milohanic, E., R. Jonquieres, P. Cossart, P. Berche, and J. L. Gaillard. 2001. The autolysin Ami contributes to the adhesion of Listeria monocytogenes to eukaryotic cells via its cell wall anchor. Molec. Microbiol. 39:1212–1214.

Milohanic, E., P. Glaser, J. Y. Coppee, L. Frangeul, Y. Vega, J. A. Vazquez-Boland, F. Kunst, P. Cossart, and C. Buchrieser. 2003. Transcriptome analysis of Listeria monocytogenes identifies three groups of genes differently regulated by PrfA. Molec. Microbiol. 47:1613–1625.

Mollenkopf, H., G. Dietrich, and S. H. Kaufmann. 2001. Intracellular bacteria as targets and carriers for vaccination. Biol. Chem. 382:521–532.

Moser, J., B. Gerstel, J. E. Meyer, T. Chakraborty, J. Wehland, and D. W. Heinz. 1997. Crystal structure of the phosphatidylinositol-specific phospholipase C from the human pathogen Listeria monocytogenes. J. Molec. Biol. 273:269–282.

Murray, E. G. D., R. E. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus Bacterium monocytogenes (n. sp.). J. Pathol. Bacteriol. 29:407–439.

Nadon, C. A., B. M. Bowen, M. Wiedmann, and K. J. Boor. 2002. Sigma B contributes to PrfA-mediated virulence in Listeria monocytogenes. Infect. Immun. 70:3948–3952.

Nair, S., C. Frehel, L. Nguyen, V. Escuyer, and P. Berche. 1999. ClpE, a novel member of the HSP100 family, is involved in cell division and virulence of Listeria monocytogenes. Molec. Microbiol. 31:185–196.

Nair, S., I. Derre, T. Msadek, O. Gaillot, and P. Berche. 2000. CtsR controls class III heat shock gene expression in the human pathogen Listeria monocytogenes. Molec. Microbiol. 35:800–811.

Navarre, W. W., and O. Schneewind. 1999. Surface proteins of Gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol. Molec. Biol. Rev. 63:174–229.

Neuhaus, F. C., and J. Baddiley. 2003. A continuum of anionic charge: Structures and functions of D-alanyl-teichoic acids in Gram-positive bacteria. Microbiol. Molec. Biol. Rev. 67:686–723.

Nishibori, T., H. Xiong, I. Kawamura, M. Arakawa, and M. Mitsuyama. 1996. Induction of cytokine gene expression by listeriolysin O and roles of macrophages and NK cells. Infect. Immun. 64:188–195.

Nomura, T., I. Kawamura, K. Tsuchiya, C. Kohda, H. Baba, Y. Ito, T. Kimoto, I. Watanabe, and M. Mitsuyama. 2002. Essential role of interleukin-12 (IL-12) and IL-18 for gamma interferon production induced by listeriolysin O in mouse spleen cells. Infect. Immun. 70:1049–1055.

North, R. J. 1969. Cellular kinetics associated with the development of acquired cellular resistance. J. Exp. Med. 130:299–314.

Okamoto, M., A. Nakane, and T. Minagawa. 1994. Host resistance to an intragastric infection with Listeria monocytogenes in mice depends on cellular immunity and intestinal bacterial flora. Infect. Immun. 62:3080–3085.

Olier, M., F. Pierre, S. Rousseaux, J. P. Lemaitre, A. Rousset, P. Piveteau, and J. Guzzo. 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.

Pandiripally, V. K., D. G. Westbrook, G. R. Sunki, and A. K. Bhunia. 1999. Surface protein p104 is involved in adhesion of Listeria monocytogenes to human intestinal cell line, Caco-2. J. Med. Microbiol. 48:117–124.

Paquet, A. J., K. M. Raines, and P. C. Brownback. 1986. Immunopotentiating activities of cell walls, peptidoglycans, and teichoic acids from two strains of Listeria monocytogenes. Infect. Immun. 54:170–176.

Parham, P., and E. R. Unanue. 1997. Immunity to Listeria monocytogenes: A model intracellular pathogen. Immunol. Rev. 158:1–169.

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

Park, S. F., and G. S. Stewart. 1990. High-efficiency transformation of Listeria monocytogenes by electroporation of penicillin treated cells. Gene 94:129–132.

Park, S. F., and R. G. Kroll. 1993. Expression of listeriolysin and phosphatidylinositol-specific phospholipase C is repressed by the plant-derived molecule cellobiose in Listeria monocytogenes. Molec. Microbiol. 8:653–661.

Pearson, L. D., and J. W. Osebold. 1973. Effects of antithymocyte and antimacrophage sera on the survival of mice infected with Listeria monocytogenes. Infect. Immun. 7:479–486.

Peerschke, E. I., K. B. Reid, and B. Ghebrehiwet. 1994. Identification of a novel 33-kDa C1q-binding site on human blood platelets. J. Immunol. 152:5896–5901.

Perkins, J. B., and P. J. Youngman. 1986. Construction and properties of Tn917-lac, a transposon derivative that mediates transcriptional gene fusions in Bacillus subtilis. Proc. Natl. Acad. Sci. USA 83:140–144.

Pileri, P., Y. Uematsu, S. Campagnoli, G. Galli, F. Falugi, R. Petracca, A. J. Weiner, M. Houghton, D. Rosa, G. Grandi, and S. Abrignani. 1998. Binding of hepatitis C virus to CD81. Science 282:938–941.

Pilgrim, S., A. Kolb-Maurer, I. Gentschev, W. Goebel, and M. Kuhn. 2003a. Deletion of the gene encoding p60 in Listeria monocytogenes leads to abnormal cell division and loss of actin-based motility. Infect. Immun. 71:3473–3484.

Pilgrim, S., J. Stritzker, C. Schoen, A. Kolb-Maurer, G. Geginat, M. J. Loessner, I. Gentschev, and W. Goebel. 2003b. Bactofection of mammalian cells by Listeria monocytogenes: Improvement and mechanism of DNA delivery. Gene Ther. 10:2036–2045.

Pinner, R. W., A. Schuchat, B. Swaminathan, P. S. Hayes, K. A. Deaver, R. E. Weaver, B. D. Plikaytis, M. Reeves, C. V. Broome, and J. D. Wenger. 1992. Role of foods in sporadic listeriosis. II: Microbiologic and epidemiologic investigation. The Listeria Study Group. JAMA 267:2046–2050.

Pirie, J. H. H. 1927. A new disease in veld rodents, "Tiger River disease". Publ. S. Afr. Inst. Med. Res. 3:163–186.

Pistor, S., T. Chakraborty, K. Niebuhr, E. Domann, and J. Wehland. 1994. The ActA protein of Listeria monocytogenes acts as a nucleator inducing reorganization of the actin cytoskeleton. EMBO J. 13:758–763.

Pistor, S., L. Grobe, A. S. Sechi, E. Domann, B. Gerstel, L. M. Machesky, T. Chakraborty, and J. Wehland. 2000. Mutations of arginine residues within the 146-KKRRK-150 motif of the ActA protein of Listeria monocytogenes abolish intracellular motility by interfering with the recruitment of the Arp2/3 complex. J. Cell. Sci. 113:3277–3287.

Pizarro-Cerda, J., R. Jonquires, E. Gouin, J. Vandekerckhove, J. Garin, and P. Cossart. 2002. Distinct protein patterns associated with Listeria monocytogenes InlA- or InlB-phagosomes. Cell. Microbiol. 4:101–115.

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

Portnoy, D. A., R. K. Tweten, M. Kehoe, and J. Bielecki. 1992. Capacity of listeriolysin O, streptolysin O, and perfringolysin O to mediate growth of Bacillus subtilis within mammalian cells. Infect. Immun. 60:2710–2717.

Potel, J. 1952. Zur granulomatosis infantiseptica. Zbl. Bakteriol. Orig. 158:329–331.

Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, and P. Courvalin. 1989. Molecular characterization of two proteins involved in the excision of the conjugative transposon Tn1545: Homologies with other site-specific recombinases. EMBO J. 8:2425–2433.

Poyart-Salmeron, C., C. Carlier, P. Trieu-Cuot, A. L. Courtieu, and P. Courvalin. 1990. Transferable plasmid-mediated antibiotic resistance in Listeria monocytogenes. Lancet 335:1422–1426.

Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, A. MacGowan, J. McLauchlin, and P. Courvalin. 1992. Genetic basis of tetracycline resistance in clinical isolates of Listeria monocytogenes. Antimicrob. Agents Chemother. 36:463–466.

Prats, N., V. Briones, M. M. Blanco, J. Altimira, J. A. Ramos, L. Dominguez, and A. Marco. 1992. Choroiditis and meningitis in experimental murine infection with Listeria monocytogenes. Eur. J. Clin. Microbiol. Infect. Dis. 11:744–747.

Premaratne, R. J., W. Lin, and E. A. Johnson. 1991. Development of an improved chemical defined minimal medium for Listeria monocytogenes. Appl. Environ. Microbiol. 57:3046–3048.

Pron, B., C. Boumaila, F. Jaubert, S. Sarnacki, J. P. Monnet, P. Berche, and J. L. Gaillard. 1998. Comprehensive study of the intestinal stage of listeriosis in a rat ligated ileal loop system. Infect. Immun. 66:747–755.

Provoda, C. J., E. M. Stier, and K. D. Lee. 2003. Tumor cell killing enabled by listeriolysin O-liposome-mediated delivery of the protein toxin gelonin. J. Biol. Chem. 278:35102–35108.

Racz, P., K. Tenner, and K. Szivessy. 1970. Electron microscopic studies in experimental keratoconjunctivitis listeriosa. I: Penetration of Listeria monocytogenes into corneal epithelial cells. Acta Microbiol. Acad. Sci. Hung. 17:221–236.

Racz, P., K. Tenner, and E. Mero. 1972. Experimental Listeria enteritis. I: An electron microscopic study of the epithelial phase in experimental Listeria infection. Lab. Inv. 26:694–700.

Raffelsbauer, D., A. Bubert, F. Engelbrecht, J. Scheinpflug, A. Simm, J. Hess, S. Kaufmann, and W. Goebel. 1998.

The gene cluster inlC2DE of Listeria monocytogenes contains additional new internalin genes and is important for virulence in mice. Molec. Gen. Genet. 260:144–158.

Ralovich, B., L. Emody, and E. Mero. 1972. Biological properties of virulent and avirulent Listeria monocytogenes strains. Acta Microbiol. Acad. Sci. Hung. 19:323–326.

Rathjen, J. P., and P. Moffett. 2003. Early signal transduction events in specific plant disease resistance. Curr. Opin. Plant Biol. 6:300–306.

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

Reglier-Poupet, H., C. Frehel, I. Dubail, J. L. Beretti, P. Berche, A. Charbit, and C. Raynaud. 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., E. Pellegrini, A. Charbit, and P. Berche. 2003b. Identification of LpeA, a PsaA-like membrane protein that promotes cell entry by Listeria monocytogenes. Infect. Immun. 71:474–482.

Reid, S. D., A. G. Montgomery, J. M. Voyich, F. R. DeLeo, B. Lei, R. M. Ireland, N. M. Green, M. Liu, S. Lukomski, and J. M. Musser. 2003. Characterization of an extracellular virulence factor made by group A Streptococcus with homology to the Listeria monocytogenes internalin family of proteins. Infect. Immun. 71: 7043–7052.

Renzoni, A., A. Klarsfeld, S. Dramsi, and P. Cossart. 1997. Evidence that PrfA, the pleiotropic activator of virulence genes in Listeria monocytogenes, can be present but inactive. Infect. Immun. 65:1515–1518.

Renzoni, A., P. Cossart, and S. Dramsi. 1999. PrfA, the transcriptional activator of virulence genes, is upregulated during interaction of Listeria monocytogenes with mammalian cells and in eukaryotic cell extracts. Molec. Microbiol. 34:552–561.

Repp, H., Z. Pamukci, A. Koschinski, E. Domann, A. Darji, J. Birringer, D. Brockmeier, T. Chakraborty, and F. Dreyer. 2002. Listeriolysin of Listeria monocytogenes forms Ca2+-permeable pores leading to intracellular Ca2+ oscillations. Cell. Microbiol. 4:483–491.

Riedo, F. X., R. W. Pinner, M. L. Tosca, M. L. Cartter, L. M. Graves, M. W. Reeves, R. E. Weaver, B. D. Plikaytis, and C. V. Broome. 1994. A point-source foodborne listeriosis outbreak: Documented incubation period and possible mild illness. J. Infect. Dis. 170:693–696.

Ripio, M. T., G. Dominguez-Bernal, M. Suarez, K. Brehm, P. Berche, and J. A. Vazquez-Boland. 1996. Transcriptional activation of virulence genes in wild-type strains of Listeria monocytogenes in response to a change in the extracellular medium composition. Res. Microbiol. 147:371–384.

Ripio, M. T., K. Brehm, M. Lara, M. Suarez, and J. A. Vazquez-Boland. 1997a. Glucose-1-phosphate utilization by Listeria monocytogenes is PrfA dependent and coordinately expressed with virulence factors. J. Bacteriol. 179:7174–7180.

Ripio, M. T., G. Dominguez-Bernal, M. Lara, M. Suarez, and J. A. Vazquez-Boland. 1997b. A Gly145Ser substitution in the transcriptional activator PrfA causes constitutive overexpression of virulence factors in Listeria monocytogenes. J. Bacteriol. 179:1533–1540.

Roberts, M. F., G. R. Jacobson, P. J. Scott, C. S. Mimura, and M. W. Stinton. 1985. 31P-NMR studies of the oral pathogen Streptococcus mutans: Observation of lipoteichoic acid. Biochim. Biophys. Acta 845:242–248.

Roberts, A. J., and M. Wiedman. 2003. Pathogen, host and environmental factors contributing to the pathogenesis of listeriosis. Cell. Molec. Life Sci. 60:904–918.

Rocourt, J., and P. Cossart. 1997. Listeria monocytogenes. *In:* B. Doyle and Montville (Eds.) Food Microbiology, Fundamentals and Frontiers. ASM Press. Washington, DC. 337–352.

Rocourt, J., P. BenEmbarek, H. Toyofuku, and J. Schlundt. 2003. Quantitative risk assessment of Listeria monocytogenes in ready-to-eat foods: The FAO/WHO approach. FEMS Immunol. Med. Microbiol. 35:263–267.

Roll, J. T., and C. J. Czuprynski. 1990. Hemolysin is required for extraintestinal dissemination of Listeria monocytogenes in intragastrically inoculated mice. Infect. Immun. 58:3147–3150.

Rose, F., S. A. Zeller, T. Chakraborty, E. Domann, T. Machleidt, M. Kronke, W. Seeger, F. Grimminger, and U. Sibelius. 2001. Human endothelial cell activation and mediator release in response to Listeria monocytogenes virulence factors. Infect. Immun. 69: 897–905.

Rouquette, C., M. T. Ripio, E. Pellegrini, J. M. Bolla, R. I. Tascon, J. A. Vazquez-Boland, and P. Berche. 1996. Identification of a ClpC ATPase required for stress tolerance and in vivo survival of Listeria monocytogenes. Molec. Microbiol. 21:977–987.

Rouquette, C., C. de Chastellier, S. Nair, and P. Berche. 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. Molec. Microbiol. 27:1235–1245.

Ruhland, G. J., and F. Fiedler. 1987. Occurrence and biochemistry of teichoic acids in the genus Listeria. Syst. Appl. Microbiology 9:40–46.

Ruhland, G. J., M. Hellwig, G. Wanner, and F. Fiedler. 1993. Cell-surface location of Listeria-specific protein p60—detection of Listeria cells by indirect immunofluorescence. J. Gen. Microbiol. 139:609–616.

Ryan, M., T. O. Zaikova, J. F. Keana, H. Goldfine, and O. H. Griffith. 2002. Listeria monocytogenes phosphatidylinositol-specific phospholipase C: Activation and allostery. Biophys. Chem. 101–102:347–358.

Saiki, I., K. Kamisango, Y. Tanio, H. Okumura, Y. Yamamura, and I. Azuma. 1982. Adjuvant activity of purified peptidoglycan of Listeria monocytogenes in mice and guinea pigs. Infect. Immun. 38:58–65.

Salamina, G., E. Dalle Donne, A. Niccolini, G. Poda, D. Cesaroni, M. Bucci, R. Fini, M. Maldini, A. Schuchat, B. Swaminathan, W. Bibb, J. Rocourt, N. Binkin, and S. Salmaso. 1996. A foodborne outbreak of gastroenteritis involving Listeria monocytogenes. Epidemiol. Infect. 117:429–436.

Samarin, S., S. Romero, C. Kocks, D. Didry, D. Pantaloni, and M. F. Carlier. 2003. How VASP enhances actin-based motility. J. Cell. Biol. 163:131–142.

Schaferkordt, S., and T. Chakraborty. 1995. Vector plasmid for insertional mutagenesis and directional cloning in Listeria spp. BioTechniques 19:720–725.

Scheld, W. M., D. D. Fletcher, F. N. Fink, and M. A. Sande. 1979. Response to therapy in an experimental rabbit model of meningitis due to Listeria monocytogenes. J. Infect. Dis. 140:287–294.

Schlech 3rd, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis—evidence for transmission by food. N. Engl. J. Med. 308:203–206.

Schlech 3rd, W. F. 1984. New perspectives on the gastrointestinal mode of transmission in invasive Listeria monocytogenes infection. Clin. Invest. Med. 7:321–324.

Schlech, W. F. 1997. Listeria gastroenteritis—old syndrome, new pathogen. N. Engl. J. Med. 336:130–132.

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

Schleifer, K.-H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. 36:407–477.

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

Schubert, K., A. M. Bichlmaier, E. Mager, K. Wolff, G. Ruhland, and F. Fiedler. 2000. P45, an extracellular 45 kDa protein of Listeria monocytogenes with similarity to protein p60 and exhibiting peptidoglycan lytic activity. Arch. Microbiol. 173:21–28.

Schubert, W. D., C. Urbanke, T. Ziehm, V. Beier, M. P. Machner, E. Domann, J. Wehland, T. Chakraborty, and D. W. Heinz. 2002. Structure of internalin, a major invasion protein of Listeria monocytogenes, in complex with its human receptor E-cadherin. Cell 111:825–836.

Schwan, W. R., A. Demuth, M. Kuhn, and W. Goebel. 1994. Phosphatidylinositol-specific phospholipase C from Listeria monocytogenes contributes to intracellular survival and growth of Listeria innocua. Infect. Immun. 62:4795–4803.

Schwartz, B., D. Hexter, C. V. Broome, A. W. Hightower, R. B. Hirschhorn, J. D. Porter, P. S. Hayes, W. F. Bibb, B. Lorber, and D. G. Faris. 1989. Investigation of an outbreak of listeriosis: New hypotheses for the etiology of epidemic Listeria monocytogenes infections. J. Infect. Dis. 159:680–685.

Schwarzer, N., R. Nost, J. Seybold, S. K. Parida, O. Fuhrmann, M. Krull, R. Schmidt, R. Newton, S. Hippenstiel, E. Domann, T. Chakraborty, and N. Suttorp. 1998. Two distinct phospholipases C of Listeria monocytogenes induce ceramide generation, nuclear factor-kappa B activation, and E-selectin expression in human endothelial cells. J. Immunol. 161:3010–3018.

Seeliger, H., and D. Jones. 1986. Genus Listeria. *In:* M. Smeath, Sarpe, and Holt (Eds.) Bergey's Manual of Systematic Bacteriology. Williams and Wilkins. Baltimore, MD. 1235–1245.

Sheehan, B., A. Klarsfeld, T. Msadek, and P. Cossart. 1995. Differential activation of virulence gene expression by PrfA, the Listeria monocytogenes virulence regulator. J. Bacteriol. 177:6469–6476.

Sheehan, B., A. Klarsfeld, R. Ebright, and P. Cossart. 1996. A single substitution in the putative helix-turn-helix motif of the pleiotropic activator PrfA attenuates Listeria monocytogenes virulence. Molec. Microbiol. 20:785–797.

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

Sibelius, U., T. Chakraborty, B. Krogel, J. Wolf, F. Rose, R. Schmidt, J. Wehland, W. Seeger, and F. Grimminger. 1996a. The listerial exotoxins listeriolysin and phosphatidylinositol-specific phospholipase C synergize to elicit endothelial cell phosphoinositide metabolism. J. Immunol. 157:4055–4060.

Sibelius, U., F. Rose, T. Chakraborty, A. Darji, J. Wehland, S. Weiss, W. Seeger, and F. Grimminger. 1996b. Listeriolysin is a potent inducer of the phosphatidylinositol response and lipid mediator generation in human endothelial cells. Infect. Immun. 64:674–676.

Sibelius, U., E. C. Schulz, F. Rose, K. Hattar, T. Jacobs, S. Weiss, T. Chakraborty, W. Seeger, and F. Grimminger. 1999. Role of Listeria monocytogenes exotoxins listeriolysin and phosphatidylinositol-specific phospholipase C in activation of human neutrophils. Infect. Immun. 67:1125–1130.

Sirard, J. C., C. Fayolle, C. de Chastellier, M. Mock, C. Leclerc, and P. Berche. 1997. Intracytoplasmic delivery of listeriolysin O by a vaccinal strain of Bacillus anthracis induces CD8-mediated protection against Listeria monocytogenes. J. Immunol. 159:4435–4443.

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

Skoble, J., V. Auerbuch, E. D. Goley, M. D. Welch, and D. A. Portnoy. 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., C. G. Gahan, T. Abee, and C. Hill. 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., C. G. Gahan, and C. Hill. 2001a. Identification and disruption of the proBA locus in Listeria monocytogenes: Role of proline biosynthesis in salt tolerance and murine infection. Appl. Environ. Microbiol. 67:2571–2577.

Sleator, R. D., J. Wouters, C. G. Gahan, T. Abee, and C. Hill. 2001b. 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., C. G. Gahan, and C. Hill. 2003. A postgenomic appraisal of osmotolerance in Listeria monocytogenes. Appl. Environ. Microbiol. 69:1–9.

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

Smith, K., and P. Youngman. 1992. Use of a new integrational vector to investigate compartment-specific expression of the Bacillus subtilis spoIIM gene. Biochimie 74:705–711.

Smith, G. A., H. Marquis, S. Jones, N. C. Johnston, D. A. Portnoy, and H. Goldfine. 1995a. 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.

Smith, G. A., D. A. Portnoy, and J. A. Theriot. 1995b. Asymmetric distribution of the Listeria monocytogenes ActA protein is required and sufficient to direct actin-based motility. Molec. Microbiol. 17:945–951.

Smith, T. J., S. A. Blackman, and S. J. Foster. 2000. Autolysins of Bacillus subtilis: Multiple enzymes with multiple functions. Microbiology 146:249–262.

Snyder, A., and H. Marquis. 2003. Restricted translocation across the cell wall regulates secretion of the broad-range phospholipase C of Listeria monocytogenes. J. Bacteriol. 185:5953–5958.

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

Southwick, F. S., and D. L. Purich. 1996. Intracellular pathogenesis of listeriosis. N. Engl. J. Med. 21:12.

Stamm, A. M., W. E. Dismukes, B. P. Simmons, C. G. Cobbs, A. Elliott, P. Budrich, and J. Harmon. 1982. Listeriosis in renal transplant recipients: Report of an outbreak and review of 102 cases. Rev. Infect. Dis. 4:665–682.

Stamm, L. M., J. H. Morisaki, L. Y. Gao, R. L. Jeng, K. L. McDonald, R. Roth, S. Takeshita, J. Heuser, M. D. Welch, and E. J. Brown. 2003. Mycobacterium marinum escapes from phagosomes and is propelled by actin-based motility. J. Exp. Med. 198:1361–1368.

Steffen, P., D. A. Schafer, V. David, E. Gouin, J. A. Cooper, and P. Cossart. 2000. Listeria monocytogenes ActA protein interacts with phosphatidylinositol 4,5-bisphosphate in vitro. Cell. Motil. Cytoskeleton 45:58–66.

Stelma, G. N., A. L. Reyes, J. T. Peeler, D. W. Francis, J. M. Hunt, P. L. Spaulding, C. H. Johnson, and J. Lovett. 1987. Pathogenicity test for Listeria monocytogenes using immunocompromised mice. J. Clin. Microbiol. 25:2085–2089.

Stockinger, S., T. Materna, D. Stoiber, L. Bayr, R. Steinborn, T. Kolbe, H. Unger, T. Chakraborty, D. E. Levy, M. Muller, and T. Decker. 2002. Production of type I IFN sensitizes macrophages to cell death induced by Listeria monocytogenes. J. Immunol. 169: 6522–6529.

Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, and P. Warrener. 2000. Complete genome sequence of Pseudomonas aeruginosa PA01, an opportunistic pathogen. Nature 406:959–964.

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

Sue, D., K. J. Boor, and M. Wiedmann. 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.

Sullivan, M. R., E. Yasbin, and F. E. Young. 1984. New shuttle vectors for B. subtilis and E. coli which allow rapid detection of inserted fragments. Gene 29:21–26.

Sun, A. N., A. Camilli, and D. A. Portnoy. 1990. Isolation of Listeria monocytogenes small-plaque mutants defective for intracellular growth and cell-to-cell spread. Infect. Immun. 58:3770–3778.

Swaminathan, B., J. Rocourt, and J. Bille. 1995. Listeria. *In:* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Ternover, and R. H. Yolken (Eds.) Manual of Clinical Microbiology. ASM Press. Washington, DC. 341–348.

Swanson, J. A., and S. C. Baer. 1995. Phagocytosis by zippers and triggers. Trends Cell Biol. 5:89–93.

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

Tang, P., C. L. Sutherland, M. R. Gold, and B. B. Finlay. 1998. Listeria monocytogenes invasion of epithelial cells requires the MEK-1/ERK-2 mitogen-activated protein kinase pathway. Infect. Immun. 66:1106–1112.

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

Thirumuruhan, R., K. Rajashankar, A. A. Fedorov, T. Dodatko, M. R. Chance, P. Cossart, and S. C. Almo. 2003. Crystal structure of PrfA, the transcriptional regulator in Listeria monocytogenes. Online: Protein Data Bank, accession code 1OMI.

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

Trieu-Cuot, P., C. Carlier, C. Poyart-Salmeron, and P. Courvalin. 1991. Shuttle vectors containing a multiple cloning site and a lacZ alpha gene for conjugal transfer of DNA from Escherichia coli to Gram-positive bacteria. Gene 102:99–104.

Troxler, R., A. von Graevenitz, G. Funke, B. Wiedemann, and I. Stock. 2000. Natural antibiotic susceptibility of Listeria species: L. grayi, L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri and L. welshimeri strains. Clin. Microbiol. Infect. 6:525–535.

Tsai, H. N., and D. A. Hodgson. 2003. Development of a synthetic minimal medium for Listeria monocytogenes. Appl. Environ. Microbiol. 69:6943–6945.

Tsolis, R. M., S. M. Townsend, E. A. Miao, S. I. Miller, T. A. Ficht, L. G. Adams, and A. J. Baumler. 1999. Identification of a putative Salmonella enterica Serotype typhimurium host range factor with homology to IpaH and YopM by signature-tagged mutagenesis. Infect. Immun. 67:6385–6393.

Vazquez, M. A., S. C. Sicher, W. J. Wright, M. L. Proctor, S. R. Schmalzried, K. R. Stallworth, J. C. Crowley, and C. Y. Lu. 1995. Differential regulation of TNF-alpha production by listeriolysin-producing versus nonproducing strains of Listeria monocytogenes. J. Leuk. Biol. 58:556–562.

Vazquez-Boland, J. A., L. Dominguez, E. F. Rodriguez-Ferri, and G. Suarez. 1989. Purification and characterization of two Listeria ivanovii cytolysins, a sphingomyelinase C and a thiol-activated toxin (ivanolysin O). Infect. Immun. 57:3928–3935.

Vazquez-Boland, J. A., C. Kocks, S. Dramsi, H. Ohayon, C. Geoffroy, J. Mengaud, and P. Cossart. 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., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzalez-Zorn, J. Wehland, and J. Kreft. 2001. Listeria pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14:584–640.

Vega, Y., C. Dickneite, M. T. Ripio, R. Bockmann, B. Gonzalez-Zorn, S. Novella, G. Dominguez-Bernal, W. Goebel, and J. A. Vazquez-Boland. 1998. Functional similarities between the Listeria monocytogenes virulence regulator PrfA and cyclic AMP receptor protein: The PrfA* (Gly145Ser) mutation increases binding affinity for target DNA. J. Bacteriol. 180: 6655–6660.

Velge, P., E. Bottreau, N. van Langendonck, and B. Kaeffer. 1997. Cell proliferation enhances entry of Listeria monocytogenes into intestinal epithelial cells by two proliferation-dependent entry pathways. J. Med. Microbiol. 46:681–692.

Vicente, M. F., F. Baquero, and J. C. Perez-Diaz. 1987. A protoplast transformation system for Listeria sp. Plasmid 18:89–92.

Virji, M., K. Makepeace, D. J. Ferguson, and S. M. Watt. 1996. Carcinoembryonic antigens (CD66) on epithelial cells and neutrophils are receptors for Opa proteins of pathogenic Neisseriae. Molec. Microbiol. 22:941–950.

Wadsworth, S. J., and H. Goldfine. 1999. Listeria monocytogenes phospholipase C-dependent calcium signaling modulates bacterial entry into J774 macrophage-like cells. Infect. Immun. 67:1770–1778.

Wadsworth, S. J., and H. Goldfine. 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.

Wampler, J. L., K. P. Kim, Z. Jaradat, and A. K. Bhunia. 2004. Heat shock protein 60 acts as a receptor for the Listeria adhesion protein in Caco-2 cells. Infect. Immun. 72:931–936.

Ward, J. B., and R. Williamson. 1984. Bacterial autolysins: Specificity and function. *In:* C. Nombela (Ed.) Microbial Wall Synthesis and Function. Elsevier. Amsterdam, The Netherlands. 159–166.

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

Weiglein, I., W. Goebel, J. Troppmair, U. R. Rapp, A. Demuth, and M. Kuhn. 1997. Listeria monocytogenes infection of HeLa cells results in listeriolysin O-mediated transient activation of the Raf-MEK-MAP kinase pathway. FEMS Microbiol. Lett. 148:189–195.

Welch, M. D., A. Iwamatsu, and T. J. Mitchison. 1997. Actin polymerization is induced by Arp2/3 protein complex at the surface of Listeria monocytogenes. Nature 385:265–269.

Welch, M. D., J. Rosenblatt, J. Skoble, D. A. Portnoy, and T. J. Mitchison. 1998. Interaction of human Arp2/3 complex and the Listeria monocytogenes ActA 3protein in actin filament nucleation. Science 281(5373):105–108.

Wemekamp-Kamphuis, H. H., J. A. Wouters, R. D. Sleator, C. G. Gahan, C. Hill, and T. Abee. 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.

Wendlinger, G., M. J. Loessner, and S. Scherer. 1996. Bacteriophage receptors on Listeria monocytogenes cells are the N-acetylglucosamine and rhamnose substituents of teichoic acids or the peptidoglycan itself. Microbiology 142:985–992.

Wing, E. W., and S. H. Gregory. 2002. Listeria monocytogenes: Clinical and experimental update. J. Infect. Dis. 185:S18–S24.

Wirth, R., F. Y. An, and D. B. Clewell. 1986. Highly efficient protoplast transformation system for Streptococcus faecalis and a new Escherichia coli-S. faecalis shuttle vector. J. Bacteriol. 165:831–836.

Wood, S., N. Maroushek, and C. J. Czuprynski. 1993. Multiplication of Listeria monocytogenes in a murine hepatocyte cell line. Infect. Immun. 61:3068–3072.

Wuenscher, M. D., S. Kohler, A. Bubert, U. Gerike, and W. Goebel. 1993. The iap gene of Listeria monocytogenes is essential for cell viability, and its gene product, p60, has bacteriolytic activity. J. Bacteriol. 175: 3491–3501.

Xiong, H., I. Kawamura, T. Nishibori, and M. Mitsuyama. 1994. Cytokine gene expression in mice at an early stage of infection with various strains of Listeria spp. differing in virulence. Infect. Immun. 62:3649–3654.

Yarar, D., W. To, A. Abo, and M. D. Welch. 1999. The Wiskott-Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex. Curr. Biol. 9:555–558.

Yoshikawa, H., I. Kawamura, M. Fujita, H. Tsukada, M. Arakawa, and M. Mitsuyama. 1993. Membrane damage and interleukin-1 production in murine macrophages exposed to listeriolysin O. Infect. Immun. 61:1334–1339.

Young, F. E. 1967. Requirement of glucosylated teichoic acid for adsorption of phage in Bacillus subtilis. Proc. Natl. Acad. Sci. USA 58(6):2377–2384.

Zachar, Z., and D. C. Savage. 1979. Microbial interference and colonization of the murine gastrointestinal tract by Listeria monocytogenes. Infect. Immun. 23:168–174.

Zhang, C., M. Zhang, J. Ju, J. Nietfeldt, J. Wise, P. M. Terry, M. Olson, S. D. Kachman, M. Wiedmann, M. Samadpour, and A. K. Benson. 2003. Genome diversification in phylogenetic lineages I and II of Listeria monocytogenes: Identification of segments unique to lineage II populations. J. Bacteriol. 185:5573–5584.

Zimmer, M., E. Sattelberger, R. B. Inman, R. Calendar, and M. J. Loessner. 2003. Genome and proteome of Listeria monocytogenes phage PSA: An unusual case for programmed +1 translational frameshifting in structural protein synthesis. Molec. Microbiol. 50:303–317.

Zink, R., M. J. Loessner, and S. Scherer. 1995. Characterization of cryptic prophages (monocins) in Listeria and sequence analysis of a holin/endolysin gene. Microbiology 141:2577–2584.