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Listeria monocytogenes σ^B Contributes to Invasion of Human Intestinal Epithelial Cells

Heesun Kim,[1] Kathryn J. Boor,[1] and Hélène Marquis[2]*

Department of Food Science[1] and Department of Microbiology and Immunology,[2] Cornell University, Ithaca, New York

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The role of σ^B in *Listeria monocytogenes* infection of human intestinal epithelial cells was investigated. Invasion defects associated with loss of σ^B paralleled those of a Δ*inlA* strain independently of the σ^B-dependent $P2_{prfA}$ promoter. Concomitantly, amounts of *inlA* transcript and InlA protein were significantly decreased in the Δ*sigB* strain.

The gram-positive facultative intracellular food-borne pathogen *Listeria monocytogenes* is associated with serious invasive infections in humans and animals (10). To establish a food-borne bacterial infection, a pathogen must survive rapidly changing environmental conditions encountered in the gastrointestinal tract, such as exposure to bile salts, organic acids, and changing osmolarity. Survival of extreme and rapidly changing conditions requires timely and appropriate alterations in bacterial gene expression and protein activity. At the transcriptional level, these alterations are often controlled by alternative sigma factors and the catalytic core of RNA polymerase (24). Alternative sigma factors differentially associate with the core polymerase and essentially reprogram promoter recognition specificities of the enzyme, thus allowing expression of new sets of target genes. For *L. monocytogenes*, the alternative sigma factor σ^B (encoded by *sigB*) contributes to bacterial resistance to environmental stress conditions, such as reduced temperature, oxidative stress, carbon starvation, and low pH (2, 3, 12, 13, 39), as well as to virulence in the mouse (29, 39).

L. monocytogenes is capable of survival and growth under widely varying environmental conditions (11). Further, this organism is able to invade and multiply in a wide range of professional and nonprofessional phagocytic mammalian cells (38). Two cell wall-anchored proteins, internalin A (InlA) and internalin B (InlB), are necessary for efficient invasion of human nonprofessional phagocytic cells (7, 16). The intracellular infectious cycle is characterized by escape from the primary vacuole, intracytosolic replication, actin-based motility, and direct spread to neighboring cells, where a new cycle initiates (17, 36). Several virulence factors responsible for these cellular events have been identified. A pore-forming hemolysin (listeriolysin O) (17), along with two phospholipases (PI-PLC and PC-PLC) (6, 37), is involved in bacterial escape from vacuoles, and the ActA surface protein is responsible for F-actin polymerization and intracellular bacterial motility (21). A DNA binding protein, PrfA, controls expression of these virulence genes (5, 8, 22, 23, 25, 33), but the precise mechanism of regulation is not well understood. *prfA* is transcribed from three independent promoters, P_{plcA}, $P1_{prfA}$, and $P2_{prfA}$ (15). PrfA has been suggested to exist in functionally active or inactive forms, depending on environmental conditions (5, 19, 27, 31, 32), and to interact differently at various target promoters (15, 40). The presence of σ^B-dependent promoters upstream of *prfA* ($P2_{prfA}$ [29]) and *inlA* ($P4_{inlA}$ [20]) suggests that σ^B contributes to *L. monocytogenes* virulence. Further, Milohanic et al. (28) have reported that a number of PrfA-regulated genes have putative σ^B-dependent promoters. In the present study, the role of σ^B in infection of human intestinal epithelial cells was investigated.

σ^B contributes to early stages of infection in human intestinal epithelial cells. Intracellular growth of the wild-type *L. monocytogenes* strain 10403S (4) as well as Δ*sigB* (FSL A1-254) (39) and $\Delta P2_{prfA}$ (DP-L1957) (14) isogenic mutant strains was examined in two intestinal epithelial cell lines, Henle-407 and Caco-2. The assay was performed as previously described (30). Briefly, bacteria were grown at 30°C overnight in brain heart infusion (BHI) broth (with shaking at 250 rpm) and washed in phosphate-buffered saline (PBS) before infection. Host cell infections were performed at 37°C in a 5% CO_2 atmosphere. Monolayers of host cells on glass coverslips were infected with a multiplicity of infection (MOI) of approximately 30 and 3 for Henle-407 and Caco-2 cells, respectively. Cells were washed at 1 h postinfection, and gentamicin was added at 1.5 h postinfection. Gentamicin concentrations were experimentally optimized to 150 μg/ml (Henle-407) or 50 μg/ml (Caco-2) to achieve effective killing of extracellular bacteria within 30 min. The number of intracellular bacteria per coverslip was determined at various time points during infection by plating appropriate serial dilutions of infected host cell lysates onto Luria-Bertani (LB) agar plates. Intracellular doubling times were evaluated by one-way analysis of variance (ANOVA), and individual comparisons were made with Bonferroni's multiple comparison test to determine whether a mean doubling time for one strain was significantly different from those for other strains. Intracellular growth rates were not significantly different among the various strains tested (Fig. 1; Table 1). However, at 2.5 h postinfection intracellular Δ*sigB* numbers were consistently 2.0- to 2.5-fold lower than those of the wild-type strain in both cell lines. Intracellular $\Delta P2_{prfA}$

* Corresponding author. Mailing address: Department of Microbiology and Immunology, VMC 5-169, Cornell University, Ithaca, NY 14853. Phone: (607) 253-3273. Fax: (607) 253-3384. E-mail: hm72@cornell.edu.

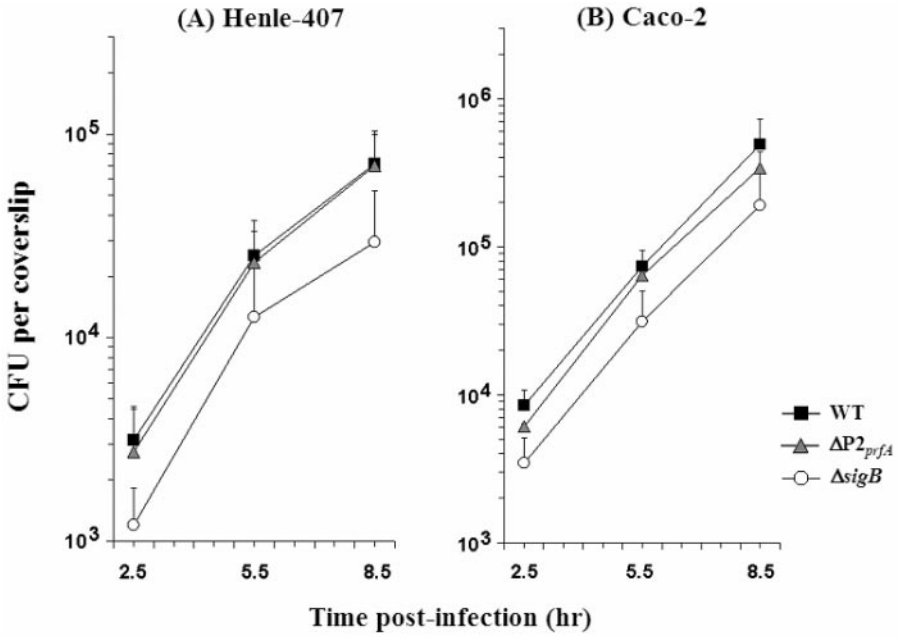

FIG. 1. Intracellular growth of *L. monocytogenes* in human epithelial cells. Viable bacterial counts were determined as CFU per coverslip at specific time points postinfection in (A) Henle-407 and (B) Caco-2 cells. Data represent mean CFU from three independent experiments. Error bars reflect standard deviations from each mean. WT, wild type.

numbers were not different than those of the wild-type strain in either cell line. These results suggest that σ^B contributes to infection independently of its activity at $P2_{prfA}$, at a stage prior to intracellular bacterial multiplication in human intestinal epithelial cells.

σ^B promotes invasion of human epithelial cells. To define the role of σ^B in early infection, we evaluated the relative abilities of the wild-type, Δ*sigB*, and $\Delta P2_{prfA}$ strains to adhere to Henle-407 and Caco-2 human epithelial cells. At 30 min prior to infection, cytochalasin D (1 μg/ml) was added to host cell monolayers to disrupt actin polymerization and prevent bacterial internalization. Bacteria were grown as described above, and cells were infected to an MOI of approximately 5. After either 1 h (Henle-407) or 30 min (Caco-2) of incubation, cells were washed six times with PBS and the number of adherent bacteria per coverslip was determined as described above. The fraction of inoculum recovered was calculated for each individual sample. To control for inhibition of bacterial internalization, a sample was treated with gentamicin after the wash and bacterial counts were determined after 1 h. No bacteria were recovered under these conditions. Results were evaluated by one-sample *t* test to determine if a given mutant strain phenotype was significantly different from that of the wild-type strain. The three bacterial strains tested did not differ in their abilities to adhere to either host cell line (Table 2).

Next we evaluated σ^B's contribution to invasion of human epithelial cells. *L. monocytogenes* invasion of nonphagocytic cells is predominantly mediated by two cell surface-anchored proteins, InlA and InlB (7, 16). The *inlA* and *inlB* genes comprise an operon. Transcriptional analysis has identified four promoters upstream of *inlA* and one upstream of *inlB* (23). $P3_{inlA}$ is a PrfA-regulated promoter (23), whereas $P4_{inlA}$ is σ^B dependent (20). A sequence resembling a σ^B-dependent promoter also has been reported upstream of *inlB*, but promoter activity at this site has not been confirmed (20). Therefore, we hypothesized that σ^B's contribution to *inlA* expression, and possibly to *inlB* expression, would influence the ability of *L.*

TABLE 1. Intracellular doubling times[a]

Strain	Doubling time (min) for Henle-407 at:		Doubling time (min) for Caco-2 at:	
	2.5–5.5 h	5.5–8.5 h	2.5–5.5 h	5.5–8.5 h
Wild type	59 ± 5	119 ± 61	58 ± 3	67 ± 5
Δ*sigB*	59 ± 11	163 ± 87	59 ± 7	70 ± 8
$\Delta P2_{prfA}$	59 ± 8	116 ± 35	54 ± 5	77 ± 12

[a] The means and standard deviations from three independent experiments are shown for each strain. At 2.5 to 5.5 h and 5.5 to 8.5 h, *P* values were 0.9934 and 0.6299 for Henle-407, respectively, and they were 0.5380 and 0.8503, respectively, for Caco-2.

TABLE 2. Adhesion and invasion characteristics of *L. monocytogenes* wild-type and mutant strains[a]

Strain	Adhesion		Invasion	
	Henle-407	Caco-2	Henle-407	Caco-2
Wild type	1.00	1.00	1.00	1.00
Δ*sigB*	0.81 ± 0.17	0.68 ± 0.20	0.54 ± 0.11***[b]	0.23 ± 0.10***
$\Delta P2_{prfA}$	0.95 ± 0.18	1.28 ± 0.33	1.08 ± 0.11	1.07 ± 0.11
Δ*inlA*	ND[c]	ND	0.52 ± 0.20**	0.21 ± 0.10**
Δ*inlB*	ND	ND	0.79 ± 0.27	0.93 ± 0.35

[a] Results are reported as ratios of mutant strain numbers relative to wild-type strain numbers, with wild-type numbers arbitrarily set to 1. The means and standard deviations from at least three independent experiments are shown for each strain.

[b] *P* values (*, <0.05; **, <0.01; ***, <0.001) for comparison between respective mutant strain and wild-type invasion by one-sample *t* test are indicated by asterisks.

[c] ND, not determined.

monocytogenes to invade nonphagocytic cells. To assess the respective contributions of σ^B, $P2_{prfA}$, InlA, and InlB to host cell invasion, invasion capabilities were compared among wild-type 10403S, Δ*sigB* (FSL A1-254) (39), $\Delta P2_{prfA}$ (DP-L1957) (14), Δ*inlA* (EJL12) (provided by Jeff Miller) (1), and Δ*inlB* (HEL-137; from this study). For this purpose, we created an *inlB* internal in-frame deletion mutant. The deletion was generated by site-directed mutagenesis by overlap extension (18), using primers Marq 37 (5′-GCG GAT CCT ACG GCA CCA ACA AAA G-3′), Marq 38 (5′-GTG GAA CTA GTC CTT ATT CGC TTC CTT CTT GGG TTG TGC-3′), Marq 39 (5′-GCA CAA CCC AAG AAG GAA GCG AAT AAG GAC TAG TTC CAC-3′), and Marq 41 (5′-GCT AGC ATG CAG TGA AAT TAT TGC TGG T-3′) (7). The *inlB* deletion was introduced into the chromosome of *L. monocytogenes* strain 10403S by allelic exchange as previously described (6), creating HEL-137. As for the intracellular growth assay, bacteria were grown at 30°C overnight (with shaking at 250 rpm) and host cell infections were performed at 37°C in a 5% CO_2 atmosphere. Host cells were infected to an MOI of approximately 30, and infection times were optimized for each cell type. For invasion of Henle-407 cells, cells were washed at 1 h postinfection and were treated with gentamicin at 1.5 h postinfection. For invasion of Caco-2 cells, cells were washed at 30 min postinfection and were treated with gentamicin at 45 min postinfection. Numbers of internalized bacteria per coverslip were determined 30 min after gentamicin treatment as described above. The fraction of inoculum recovered was calculated for each individual sample. Results were statistically evaluated by one-sample *t* test. The Δ*sigB* strain was less effective at invading Henle-407 ($P < 0.001$) and Caco-2 cells ($P < 0.001$) than the wild-type strain (Table 2). The invasion defect of the Δ*sigB* strain was comparable to that of the Δ*inlA* strain ($P = 0.791$ for Henle-407, $P = 0.784$ for Caco-2), but it was significantly more pronounced than that of the Δ*inlB* strain ($P = 0.029$ for Henle-407, $P = 0.001$ for Caco-2). Specifically, relative invasion capabilities of the Δ*sigB*, Δ*inlA*, and Δ*inlB* strains were reduced by 1.9-, 1.9-, and 1.3-fold, respectively, in Henle-407 cells and by 4.3-, 4.8-, and 1.1-fold, respectively, in Caco-2 cells. Invasion phenotypes of $\Delta P2_{prfA}$ and wild-type strains did not differ. Overall, these results show that σ^B significantly increases the efficacy of *L. monocytogenes* to invade human epithelial cells, independent of $P2_{prfA}$ transcriptional activity and of InlB. It is possible that σ^B influences InlB-dependent invasion in other cell types, such as hepatocytes or fibroblasts cells (7).

σ^B contributes to *inlA* expression. The above observations led us to focus on InlA as an important factor affecting *L. monocytogenes* virulence in a σ^B-dependent manner. One of the key early events in human listeriosis is bacterial entry into the intestinal epithelium. A number of studies have shown that InlA is a major bacterial ligand involved in this process (9, 16, 23, 26). Therefore, levels of *inlA* expression in wild-type, Δ*sigB*, and $\Delta P2_{prfA}$ strains were determined by semiquantitative reverse transcription-PCR (RT-PCR). Bacteria were grown with shaking (250 rpm) in BHI broth overnight at 30°C. Total RNA was isolated with the RNeasy Midi kit (Qiagen, Valencia, Calif.) according to the manufacturer's protocol. Purified RNA was quantified by absorbance at 260 nm immediately before use, and RT was performed with the Superscript First-Strand

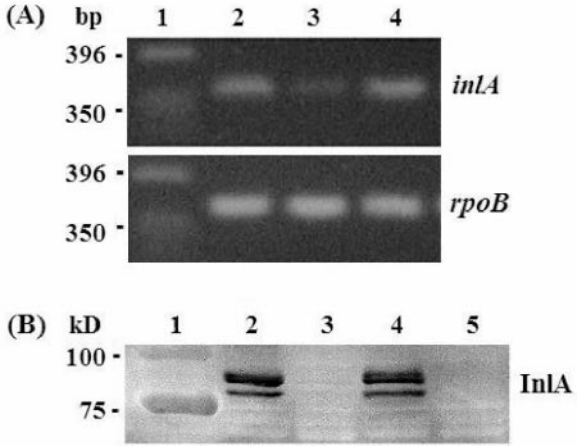

FIG. 2. Detection of *inlA* transcript and InlA protein. (A) Total RNA was isolated from wild-type *L. monocytogenes* as well as from Δ*sigB* and $\Delta P2_{prfA}$ strains, and *inlA* transcript levels were assayed by RT-PCR. *rpoB*, encoding the β subunit of the DNA-dependent RNA polymerase, was used as an internal control. No PCR products were obtained in the absence of RNA (data not shown). Lane 1, DNA markers in base pairs (bp); lane 2, wild type; lane 3, Δ*sigB*; lane 4, $\Delta P2_{prfA}$. (B) Cell wall proteins were isolated from wild-type *L. monocytogenes* as well as from Δ*sigB*, $\Delta P2_{prfA}$, and Δ*inlA* strains, and InlA was detected by Western immunoblotting. Samples were normalized for equal CFU (7.5×10^8) from each original culture. Lane 1, prestained protein markers; lane 2, wild type; lane 3, Δ*sigB*; lane 4, $\Delta P2_{prfA}$; lane 5, Δ*inlA*. kD, kilodaltons.

Synthesis RT-PCR system (Invitrogen, Carlsbad, Calif.) with 50 ng of total RNA as template (35). The *rpoB* gene was used as an internal control. Primers used in this assay (InlATqMnF1 [5′-GGT CTC ACA AAC AGA TCT AGA CCA AGT-3′], InlATqMnR1 [5′-TCA AGT ATT CCA CTC CAT CGA TAG ATT-3′], RpoBTqMnF1, and RpoBTqMnR1 [35]) were designed with PrimerExpress software (Applied Biosystems, Foster City, Calif.). Briefly, cDNA was synthesized with oligonucleotides specific for *inlA* (InlATqMnR1) and *rpoB* (RpoBTqMnR1) and then amplified by PCR using the AmpliTaq Gold DNA polymerase system (Applied Biosystems). Thirty PCR cycles were selected for cDNA amplification, as no visible products were observed after either 15 or 22 cycles. RT-PCR products were run on 3% agarose gels and quantified with LabImage software (Kapelan, Halle, Germany). Results were statistically analyzed by one-sample *t* test. RT-PCR results clearly showed decreased levels of *inlA* transcript in the Δ*sigB* strain relative to that in the wild-type strain (Fig. 2A). In the absence of σ^B, *inlA* expression was decreased by 3.8-fold ($P = 0.001$), which is consistent with the relative invasion defect observed for the Δ*sigB* strain in human epithelial cells. Quantities of the *inlA* transcript in the $\Delta P2_{prfA}$ strain were essentially identical to those of the wild-type strain ($P = 0.212$), which is also consistent with the invasion phenotype of this strain in human epithelial cells.

Levels of cell wall-associated InlA in wild-type, Δ*sigB*, and $\Delta P2_{prfA}$ strains were estimated by Western immunoblotting. For isolation of *L. monocytogenes* cell wall proteins, bacterial cells in protoplast buffer (0.5 M sucrose, 50 mM Tris-HCl [pH 8.0], 10 mM $MgCl_2$, 0.02% sodium azide) were treated with mutanolysin to a final concentration of 100 U/ml of original bacterial culture at an OD_{600} of 1.0. Protoplast formation was

monitored by microscopy. Cell wall extracts were processed for detection of InlA by Western immunoblotting as previously described (34). InlA was virtually undetectable in the Δ*sigB* strain (Fig. 2B). On the other hand, equivalent amounts of InlA were present in the wild-type and $\Delta P2_{prfA}$ strains (Fig. 2B). Based on the observations that *inlA* transcription is regulated in a growth-phase-dependent manner and that *inlA* expression peaks upon entry into stationary phase in the absence of PrfA, Sheehan et al. (33) suggested the existence of a PrfA-independent mechanism that contributes to regulation of *inlA* expression in stationary-phase *L. monocytogenes*. Our data suggest that this proposed stationary-phase mechanism is mediated by σ^B. σ^B activity is also regulated in a growth-phase-dependent manner, with maximal activity upon entry into stationary phase (13, 35). Most importantly, we have demonstrated that σ^B is a major contributor to stationary-phase *inlA* expression. Taken together, our observations highlight the critical contribution of σ^B to expression of *inlA* and consequently to invasion of human intestinal epithelial cells.

In conclusion, we have identified a specific mechanism linking the general stress-responsive sigma factor, σ^B, with the ability of *L. monocytogenes* to invade human epithelial cells. Specifically, we have demonstrated that the loss of σ^B significantly impairs the ability of *L. monocytogenes* to invade human epithelial cells in a manner that is independent of the σ^B-dependent promoter $P2_{prfA}$. Moreover, our data clearly demonstrate that the importance of σ^B in bacterial invasion is related, at least in part, to its role in the regulation of *inlA* expression, presumably by controlling activity at $P4_{inlA}$.

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REFERENCES

1. **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.
2. **Becker, L. A., M. S. Cetin, R. W. Hutkins, and A. K. Benson.** 1998. Identification of the gene encoding the alternative sigma factor σ^B from *Listeria monocytogenes* and its role in osmotolerance. J. Bacteriol. **180:**4547–4554.
3. **Becker, L. A., S. N. Evans, R. W. Hutkins, and A. K. Benson.** 2000. Role of σ^B in adaptation of *Listeria monocytogenes* to growth at low temperature. J. Bacteriol. **182:**7083–7087.
4. **Bishop, D. K., and D. J. Hinrichs.** 1987. Adoptive transfer of immunity to *Listeria monocytogenes*. The influence of in vitro stimulation on lymphocyte subset requirements. J. Immunol. **139:**2005–2009.
5. **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. Mol. Microbiol. **20:**1189–1198.
6. **Camilli, A., L. G. Tilney, and D. A. Portnoy.** 1993. Dual roles of *plcA* in *Listeria monocytogenes* pathogenesis. Mol. Microbiol. **8:**143–157.
7. **Dramsi, S., I. Biswas, E. Maguin, L. Braun, P. Mastroeni, and P. Cossart.** 1995. Entry of *Listeria monocytogenes* into hepatocytes requires expression of InlB, a surface protein of the internalin multigene family. Mol. Microbiol. **16:**251–261.
8. **Dramsi, S., C. Kocks, C. Forestier, and P. Cossart.** 1993. Internalin-mediated invasion of epithelial cells by *Listeria monocytogenes* is regulated by the bacterial growth state, temperature and the pleiotropic activator *prfA*. Mol. Microbiol. **9:**931–941.
9. **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.
10. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55:**476–511.
11. **Fenlon, D. R.** 1999. *Listeria monocytogenes* in the natural environment, p. 21–37. *In* E. T. Ryser and E. H. Marth (ed.), Listeria, listeriosis, and food safety, 2nd ed. Marcel Dekker, Inc., New York, N.Y.
12. **Ferreira, A., C. P. O'Byrne, and K. J. Boor.** 2001. Role of σ^B in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes.* Appl. Environ. Microbiol. **67:**4454–4457.
13. **Ferreira, A., D. Sue, C. P. O'Byrne, and K. J. Boor.** 2003. Role of *Listeria monocytogenes* σ^B in survival of lethal acidic conditions and in the acquired acid tolerance response. Appl. Environ. Microbiol. **69:**2692–2698.
14. **Freitag, N. E., and D. A. Portnoy.** 1994. Dual promoters of the *Listeria monocytogenes prfA* transcriptional activator appear essential in vitro but are redundant in vivo. Mol. Microbiol. **12:**845–853.
15. **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.
16. **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.
17. **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.
18. **Ho, S. N., H. D. Hunt, R. M. Horton, J. K. Pullen, and L. R. Pease.** 1989. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene **77:**51–59.
19. **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.
20. **Kazmierczak, M. J., S. C. Mithoe, K. J. Boor, and M. Wiedmann.** 2003. *Listeria monocytogenes* σ^B regulates stress response and virulence functions. J. Bacteriol. **185:**5722–5734.
21. **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.
22. **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.
23. **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.
24. **Loewen, P. C., and R. Hengge-Aronis.** 1994. The role of the sigma factor σ^S (KatF) in bacterial global regulation. Annu. Rev. Microbiol. **48:**53–80.
25. **Mengaud, J., S. Dramsi, E. Gouin, J. A. Vazquez-Boland, G. Milon, and P. Cossart.** 1991. Pleiotropic control of *Listeria monocytogenes* virulence factors by a gene that is autoregulated. Mol. Microbiol. **5:**2273–2283.
26. **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.
27. **Milenbachs, A. A., D. P. Brown, M. Moors, and P. Youngman.** 1997. Carbon-source regulation of virulence gene expression in *Listeria monocytogenes.* Mol. Microbiol. **23:**1075–1085.
28. **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. Mol. Microbiol. **47:**1613–1625.
29. **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.
30. **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.
31. **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.
32. **Ripio, M. T., K. Brehm, M. Lara, M. Suarez, and J. A. Vazquez-Boland.** 1997. Glucose-1-phosphate utilization by *Listeria monocytogenes* is PrfA dependent and coordinately expressed with virulence factors. J. Bacteriol. **179:**7174–7180.
33. **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.
34. **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.
35. **Sue, D., K. J. Boor, and M. Wiedmann.** 2003. σ^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.
36. **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.
37. **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.
38. **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.
39. **Wiedmann, M., T. J. Arvik, R. J. Hurley, and K. J. Boor.** 1998. General stress transcription factor σ^B and its role in acid tolerance and virulence of *Listeria monocytogenes.* J. Bacteriol. **180:**3650–3656.
40. **Williams, J. R., C. Thayyullathil, and N. E. Freitag.** 2000. Sequence variations within PrfA DNA binding sites and effects on *Listeria monocytogenes* virulence gene expression. J. Bacteriol. **182:**837–841.

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