INFECTION AND IMMUNITY, May 2006, p. 2505–2512 Vol. 74, No. 5
0019-9567/06/$08.00+0 doi:10.1128/IAI.74.5.2505–2512.2006

MINIREVIEW

How the Bacterial Pathogen *Listeria monocytogenes* Mediates the Switch from Environmental Dr. Jekyll to Pathogenic Mr. Hyde

Michael J. Gray,[1]† Nancy E. Freitag,[2] and Kathryn J. Boor[1]*

Department of Food Science, Cornell University, Ithaca, New York,[1] and Seattle Biomedical Research Institute and the Departments of Pathobiology and Microbiology, University of Washington, Seattle, Washington[2]

Listeria monocytogenes is a gram-positive bacterium with a Jekyll and Hyde personality (108): it is well adapted as a saprophyte for peaceful survival in soil and decaying vegetation (Dr. Jekyll) (36), but it has a second life as an intracellular bacterial pathogen capable of causing serious infection in humans and in many animal species (Mr. Hyde) (28, 96, 115). In its Mr. Hyde phase, the bacterium is a significant public health hazard, responsible for an estimated 28% of deaths attributable to known food-borne pathogens in the United States (75). How does *L. monocytogenes* manage the switch between mild-mannered environmental bacterium and potentially deadly human pathogen? The transformation appears to be mediated through complex regulatory pathways that modulate the expression of virulence factors in response to environmental cues. This review will summarize the current understanding of *L. monocytogenes* virulence gene regulation and will put forth a model that depicts how a humble soil-grown bacterium might transform into a deadly invader.

LIFE IN THE SOIL: THE PEACEFUL EXISTENCE OF A BACTERIAL DR. JEKYLL

L. monocytogenes is a ubiquitous bacterium that sets up home in a variety of environmental locations. *L. monocytogenes* has been isolated from soil, ground water, silage, and decaying vegetation (reviewed in reference 36); however, relatively little is known about the bacterium's potentially peaceful Dr. Jekyll existence. Genome sequencing indicates the presence of multiple gene products that may facilitate the utilization by *L. monocytogenes* of a variety of carbon sources, including plant sugars (48, 84). To access nutrient sources, *L. monocytogenes* expresses flagella and exhibits swimming motility at temperatures below 30°C; in many strains (but not all) swimming motility is repressed at 37°C (51, 87, 117). Although *L. monocytogenes* does not form spores, the bacterium is well known for its ability to withstand a variety of environmental stresses, including low temperature and high osmolarity (99), thus making it a hardy environmental organism.

It is possible and, perhaps, probable that the existence of *L. monocytogenes* outside of mammalian host cells is not entirely a quiet and sedate country life but, rather, a constant territorial battle with other single-cell and multicellular organisms that are lurking nearby. Although it is commonly isolated from environmental sources (36), *L. monocytogenes* maintains an arsenal of gene products that appear to be designed to facilitate survival within mammalian host cells. Maintenance of this arsenal in an organism that is broadly present in the environment suggests the possibility that these gene products may be utilized not only in mammals but also against other eukaryotic organisms in the environment. For example, while protozoa have not been reported as a reservoir for *L. monocytogenes*, as is the case with *Legionella pneumophila*, *L. monocytogenes* does survive and replicate within amoebae (49, 70, 111). *L. monocytogenes* is an efficient pathogen of at least one insect species (*Drosophila melanogaster*), although infections must be systemically induced (73). It is likely that further studies will identify additional nonmammalian organisms that serve as hosts for *L. monocytogenes*.

L. MONOCYTOGENES WITHIN MAMMALIAN HOSTS: A BACTERIAL MR. HYDE

Although *L. monocytogenes* is well adapted to persistence in the environment (36), the majority of studies focused on *L. monocytogenes* have investigated infection of mammalian hosts, or the Mr. Hyde phase of the organism: the invasion and survival within mammalian host cells and the immune response to bacterial infection (reviewed in references 28, 66, and 86). *L. monocytogenes* is capable of invading and replicating within a wide range of animal cell types, including macrophages and nonprofessional phagocytes (45, 64, 71, 89). A number of bacterial gene products have been identified that facilitate the intracellular growth and spread of the bacterium to adjacent host cells (88, 90), and the functions of these gene products have been discussed in several excellent recent reviews (20, 28, 57, 63, 115). Briefly, these gene products include the invasion-associated surface proteins internalin A and B (InlA and InlB), gene products associated with escape from the host cell vacuole (the *hly*-encoded cholesterol-dependent cytolysin listeriolysin O [LLO] as well as phospholipases encoded by *plcA* and *plcB*), and ActA, a protein required for actin-based intracellular bacterial motility and cell-to-cell spread. Additional gene products, such as Mpl, a zinc-dependent metalloprotease that processes PlcB to its mature form, and Hpt, a hexose phosphate transporter that allows bacteria to utilize phosphorylated

* Corresponding author. Mailing address: Department of Food Science, Cornell University, 413 Stocking Hall, Ithaca, NY 14853. Phone: (607) 255-3111. Fax: (607) 254-4868. E-mail: kjb4@cornell.edu.
† Present address: Department of Bacteriology, University of Wisconsin, Madison, WI 53706.

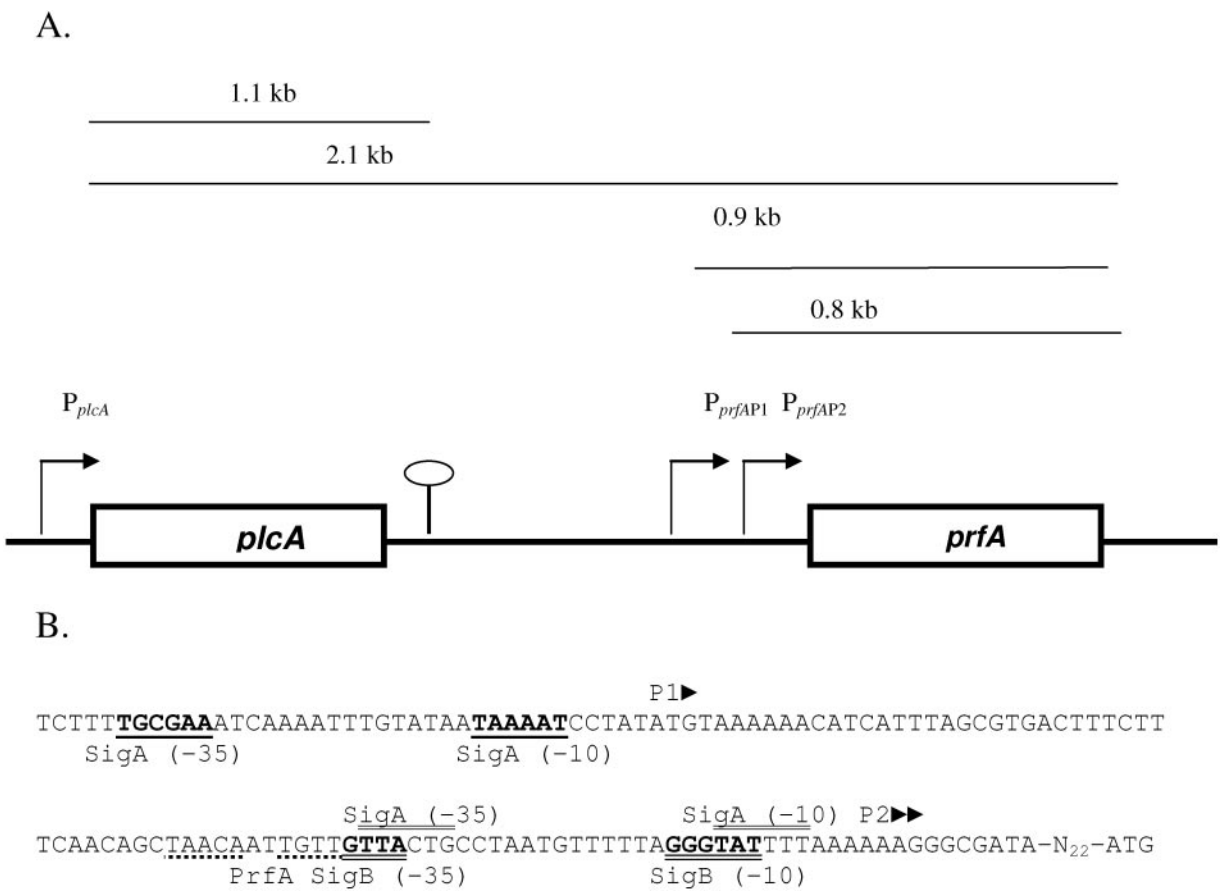

FIG. 1. (A) Map of the *L. monocytogenes plcA-prfA* region, not drawn to scale. The heavy line at the bottom of the panel represents the DNA sequence, with gene coding regions indicated as boxes; the four lines above the gene coding region represent possible mRNA transcripts, including a 1.1-kb *plcA* transcript, a 2.1-kb *plcA-prfA* bicistronic transcript, and 0.9- and 0.8-kb *prfA* transcripts (11, 13, 42, 67, 73, 91). Transcriptional start sites are indicated by bent arrows, and the *plcA* transcription terminator is indicated by a stem-loop. (B) DNA sequence of the *prfA* promoter region (46, 91). Triangles indicate transcriptional start sites identified for P1*prfA* and P2*prfA*. The σ^A-dependent P1*prfA* promoter is in boldface and underlined once. Two adjacent transcriptional start sites, possibly reflecting transcription from either the σ^A-dependent or σ^B-dependent promoter comprising the P2*prfA* region, are marked by triangles (46). In the P2*prfA* region, the σ^B-dependent promoter is in boldface and underlined twice; the proposed σ^A-dependent promoter is marked by double lines above the sequence, and the PrfA binding box (100), which is immediately upstream of the P2 promoter region, is marked by a dotted line beneath the sequence.

sugars such as glucose-1-phosphate within the host cell cytosol, also contribute to bacterial life within the mammalian host cell. Other gene products, such as the bile salt hydrolase encoded by *bsh* (4, 27) and the bile exclusion locus *bilE* (104), may function to promote bacterial survival in the liver or in extracellular environments within the mammalian host, such as the small intestine or within the gall bladder (52). For nearly every gene product identified thus far as contributing to *L. monocytogenes* survival within the host, gene expression is regulated by a transcriptional activator known as positive regulatory factor A (PrfA) (12, 40, 76, 78). Strains lacking functional PrfA are highly attenuated in animal models of infection and are forever locked into a docile and nonthreatening state.

As *L. monocytogenes* is clearly capable of adapting to multiple environments, including those outside as well as inside host cells, it is important to ask what the mechanisms are that control the switch that changes *L. monocytogenes* from a quiet soil bacterium to a ruthless invader. At least some of the answers appear to lie in the regulation of the key regulatory protein PrfA and include both the regulation of *prfA* transcription and PrfA protein activity.

INITIAL CONTROL OF THE BACTERIAL BEAST: REGULATION OF *prfA* EXPRESSION

Transcriptional control of *prfA* is the first mechanism used by *L. monocytogenes* to regulate the expression of its virulence gene products. Three promoter regions have been identified that contribute to the regulation of *prfA* expression (Fig. 1A). Two promoter regions, P$prfA_{P1}$ and P$prfA_{P2}$, are located just upstream of the *prfA* coding sequence and direct the expression of monocistronic *prfA* transcripts. The upstream *plcA* promoter (P*plcA*) directs both a monocistronic *plcA* transcript and a bicistronic transcript encoding *plcA* and *prfA* (11).

Transcription of DNA in bacteria is driven by RNA polymerase, whose specificity is determined by regulatory proteins known as sigma (σ) factors (reviewed in reference 50). The primary sigma factor determining RNA polymerase specificity

in actively growing, unstressed cells is σ^A (50, 81). The P*prfA*$_{P1}$ promoter has characteristics of a σ^A-dependent promoter (83). Transcripts are produced from this promoter by actively growing *L. monocytogenes* in broth culture. The RNA transcript of *prfA* directed by P*prfA*$_{P1}$ contains a thermosensitive structure that inhibits translation of PrfA at temperatures lower than 30°C but melts at higher temperatures, allowing translation (56). The reduced efficiency of PrfA translation at low temperatures may explain the reduced transcription of PrfA-dependent genes observed at low temperatures in broth culture. The production of monocistronic *prfA* transcript is independent of temperature, while bicistronic *plcA-prfA* transcript, which is dependent on PrfA activation, is only produced at higher temperatures (67). The presence of a pool of untranslated *prfA* transcripts may allow rapid synthesis of PrfA following infection of warm-blooded mammalian and avian host organisms, which generally have temperatures higher than the surrounding environment. Temperature regulation of bacterial protein levels is not unique to *L. monocytogenes* PrfA. To illustrate, the *Escherichia coli* heat shock response associated with σ^{32} is dependent upon the presence of a pool of untranslated *rpoH* mRNA to achieve rapid increases in σ^{32} under increased temperature conditions (82, 109).

A second *prfA* promoter region, P*prfA*$_{P2}$, also directs monocistronic *prfA* transcripts (42) (Fig. 1A and B). The P2*prfA* region contains a putative PrfA binding box, which provides an autoregulatory loop (42, 100). The P2*prfA* region comprises both a σ^A- and a σ^B-dependent promoter (91). σ^B-Dependence of the P*prfA*$_{P2}$ promoter has been demonstrated (83, 91, 98). RNA polymerase complexed with σ^B recognizes the promoters of a number of genes whose products contribute to the ability of *L. monocytogenes* to withstand environmental stresses including low pH, high osmolarity, oxidative stress, and carbon starvation (3, 15, 37, 38, 39, 58, 118, 119). The products of a number of stress response genes have been implicated in virulence; these genes include *bsh*, whose product is important for resisting the stresses imposed by exposure to bile salts (27), the *gad* system, involved in resisting acid shock (22), and *hfq*, a general stress response gene involved in resistance to osmotic and ethanol stress (15). Transcription of the invasion-associated internalin genes *inlA* and *inlB* is also influenced by σ^B (59, 60). *L. monocytogenes* cells exposed to environmental stress conditions (specifically, 0.3 M NaCl or growth to stationary phase) show a relative increase in monocistronic *prfA* transcripts initiated from P*prfA*$_{P2}$ (M. Kazmierczak, M. Wiedmann, and K. J. Boor, submitted for publication). As the P*prfA*$_{P2}$-directed message does not contain the thermosensitive RNA secondary structure present in P*prfA*$_{P1}$-directed messages (56), translation of the P*prfA*$_{P2}$ transcript may thus account for the observed expression of PrfA in some low-temperature environments, such as the cytosol of insect cells, where PrfA-dependent gene products are expressed and functional (13, 26, 73).

Finally, bicistronic *plcA-prfA* transcripts are produced from the upstream PrfA-dependent P*plcA* promoter (Fig. 1A) (11, 13, 73). PrfA thereby upregulates its own production, and this autoregulation is required for bacterial cell-to-cell spread within tissue culture cells and for bacterial virulence in animal models of infection (11, 41).

ADDITIONAL CONTROL OF THE BACTERIAL BEAST: REGULATION OF PrfA ACTIVITY

In addition to the existence of transcriptional and posttranscriptional mechanisms that control *prfA* expression and translation, PrfA activity is controlled on a posttranslational level. PrfA is a member of the Crp/Fnr transcription regulator family (65, 112). As a group, Crp/Fnr regulators respond to a broad array of signals, both intracellular and exogenous, such as the presence of small molecular cofactors (e.g., cyclic AMP for Crp) (53), as well as changes in redox potential, oxygen availability, or temperature (reviewed in reference 62). Mutants of Crp, known as Crp*, have been identified that contain amino acid substitutions that appear to lock the protein into a constitutively active form, even in the absence of the signal molecule, cyclic AMP (53). Ripio et al. (95) were the first to describe a similar mutation in PrfA (PrfA G145S, or PrfA*), which was identified in an *L. monocytogenes* strain (NCTC 7973) that constitutively expressed high levels of PrfA-dependent gene products. Recent evidence suggests that the PrfA G145S mutation may stabilize the helix-turn-helix motif relative to that of the wild-type PrfA to enhance the protein's DNA-binding affinity (29). Since the identification of PrfA G145S, additional PrfA mutations have been identified that also appear to result in a constitutively activated form of the protein (PrfA I45S, PrfA E77K, PrfA L140F, and PrfA G155S) (54, 103, 116, 121). Interestingly, recent data suggest that strains containing PrfA* mutations may be locked into a Mr. Hyde state that can increase bacterial virulence in animal models (103). For example, strains containing the PrfA G155S mutation were approximately fivefold more virulent than wild-type strains following intravenous injection of mice (103).

It is clear that PrfA exists in high- and low-activity states, with the transitions between activation states occurring in response to environmental signals; however, the nature of the potential small molecule cofactor bound by PrfA (or PrfA posttranslational modification) that triggers PrfA activation is not yet known (65, 92). A number of environmental conditions influence the expression of PrfA-dependent gene products (106). Growth in rich medium or in medium supplemented with readily metabolized carbohydrates (such as glucose, fructose, maltose, or cellobiose) inhibits transcription of PrfA-dependent virulence genes (*hly*, *plcA*, *plcB*, *mpl*, and *actA*) without affecting PrfA protein levels (32, 77). Repression of virulence gene expression by cellobiose, a common carbohydrate in plant materials but not in animal hosts, appears to be mediated by at least three different mechanisms (5, 6, 9, 55, 69, 77). In contrast to the repression of virulence gene expression by these readily metabolized sugars, the presence of phosphorylated sugars, such as glucose-1-phosphate, supports bacterial growth with no repression of PrfA-dependent virulence gene expression (94). Phosphorylated sugars present within the cytosol of mammalian host cells are postulated to serve as molecular cues signaling the opportunity for rapid intracellular *L. monocytogenes* growth (14).

Other environmental signals are known to influence virulence gene expression in *L. monocytogenes*. PrfA-dependent LLO production and *actA* expression are both activated in iron-depleted medium (17, 23). As free iron levels are extremely low in mammalian host cells ($\sim 10^{-18}$ M) (68), avail-

able iron may serve as a cue used by *L. monocytogenes* to assess its location. It is well established that expression of PrfA-dependent genes increases following treatment of the culture medium with activated charcoal (31, 32, 47, 93). Ermolaeva et al. (33) have presented evidence to suggest that activated charcoal acts by absorbing a small diffusible autorepressor molecule which *L. monocytogenes* produces during exponential growth. This strategy is reminiscent of quorum sensing mechanisms used in other bacteria to regulate genes in a bacterial cell concentration-dependent fashion (1, 34), but whether this form of virulence gene repression occurs in *L. monocytogenes* remains undetermined. In summary, several environmental conditions have been shown to influence virulence gene expression, presumably by influencing the state of PrfA activation, but the molecular mechanism responsible for the conversion of PrfA to its fully active state remains unknown.

THE TRANSITION TO MR. HYDE FOLLOWING BACTERIAL INVASION OF THE MAMMALIAN HOST

Animals have a wide array of defense mechanisms specifically designed to prevent pathogenic bacteria from settling in and making themselves at home. Once *L. monocytogenes* is ingested by a mammalian host organism, its survival within that host depends upon the bacterium's ability to withstand a number of defense mechanisms. Exposure to stresses imposed by host defense mechanisms may actually help prepare *L. monocytogenes* for its Mr. Hyde existence. Specifically, accumulating evidence suggests that environmental stress conditions encountered during passage through the stomach to the gut contribute to the infectious life cycle of *L. monocytogenes* (18, 19, 21, 74, 85, 97). For example, one early host defense encountered by *L. monocytogenes* following ingestion is the low pH environment of the stomach. A clear connection has been established between acid tolerance and virulence in *L. monocytogenes*, in that mutants with increased acid tolerance show increased virulence in mice (85), and decreased acid tolerance is correlated with decreased virulence (21, 74). The genetic mechanism(s) of this effect is not well understood, but preadaptation of *L. monocytogenes* (by exposure to pH 4.5 to 5.5, similar to the pH found in the stomach after eating [22]) increases the invasiveness of the bacteria in cell culture (18) as well as bacterial survival following macrophage infection (18, 19, 44) and following intragastric inoculation of mice (97).

The acid tolerance response of *L. monocytogenes* is at least partially dependent on σ^B (37, 38, 119), and at low pH, the production of monocistronic *prfA* transcript is strongly increased; this transcript accumulation may serve to prime the bacterium for its responses to subsequent host environments (19). As expression of a variety of stress response genes and invasion-associated internalins is regulated by the stress-responsive σ^B (58, 59, 60), the predicted net effect of *L. monocytogenes* passage through the stomach and intestine may be an increase in the production of a variety of proteins important for invasion and infection. Indeed, recent data indicate that σ^B plays a critical role during the gastrointestinal stage of listeriosis in guinea pigs (46). While passage of *L. monocytogenes* through the gut clearly is not essential for virulence, as infections in animals can be established by intraperitoneal or intravenous injection (11, 14, 27, 30, 44, 71, 119), it may increase the efficiency of infection under natural conditions.

THE MAMMALIAN CYTOSOL AND THE FULL UNLEASHING OF MR. HYDE

When *L. monocytogenes* leaves the lumen of the intestine and enters a host cell, it once again encounters several changes in its immediate environment. In contrast to the relatively high available iron and carbohydrate levels in the intestinal lumen, the phagocytic vacuole is postulated to have low quantities of available iron and carbohydrates. Low iron and low carbohydrate concentrations activate transcription from some PrfA-dependent virulence gene promoters (8, 16, 17, 32, 77). Exposure of *L. monocytogenes* to H_2O_2 increases transcription of *prfA* and *hly*, suggesting that the presence of reactive oxygen intermediates, such as those generated in activated macrophages, also may up-regulate virulence gene expression (72). The phagocytic vacuole of a macrophage rapidly becomes acidified to a pH of approximately 5.5 to 6 (2). Hence, following engulfment, the *L. monocytogenes* invader is subjected to multiple rapid environmental changes, including exposure to oxygen radicals, reduced pH, and reduced nutrient density.

Some PrfA-dependent gene products have clearly targeted roles within specific cellular locations and are differentially expressed depending upon their cellular location (10). For example, *actA* expression is primarily confined to the host cell cytosol, where it directs actin polymerization (10, 43, 80). Differential expression of PrfA-dependent promoters is influenced by sequence variations within a promoter region's PrfA box, with relative activation reflecting the similarity of a given promoter's PrfA box to the PrfA-box consensus sequence (24, 100, 120). The promoters with perfect PrfA-box sequences, P*hly* and P*plcA*, are the most efficiently transcribed and produce transcripts at relatively low PrfA concentrations (100).

Activation of transcription from the *plcA* promoter by PrfA initiates an important regulatory circuit within the host by which PrfA upregulates its own production (11, 76) and produces an increase in PrfA concentrations to enable the bacteria to establish themselves in a host cell and to move to infect new cells. Mutants that produce very small amounts of PrfA are capable of escaping from vacuoles but not of polymerizing actin or spreading between host cells (41). Cell-to-cell spread is mediated by the actin nucleating protein ActA (61, 87, 113), which is transcribed from two promoters, P*actA* and P*mpl* (61, 114). These promoters each have a single mismatched base in their PrfA boxes; therefore, transcription activation from these promoters requires an increased concentration of PrfA, such as that produced by *L. monocytogenes* present in host cytosol (100). PC-PLC, the product of the *plcB* gene, is also produced by transcription from P*actA* and P*mpl* (61, 114), and its production is important for efficient bacterial cell-to-cell spread, as it permits bacterial escape from the secondary vacuoles created when an *L. monocytogenes* cell moves into a neighboring host cell (105, 114). Two additional PrfA-dependent genes that contribute to virulence, *inlC* and *hpt*, also have single mismatches in their PrfA boxes (14, 30, 74). *inlC* encodes a small, secreted protein called internalin C (30). Expression of *inlC* is enhanced in the cytoplasm of mammalian cells (10, 30), and Δ*inlC* mutants have reduced virulence in mice (30). The

function of internalin C has not yet been fully established, although recent results show that it supports internalin A in stimulating invasion of mammalian cells (7). The *hpt* gene encodes a hexose phosphate transporter which allows *L. monocytogenes* to grow using phosphorylated sugars such as glucose-1-phosphate as a carbon source (14). Deletion of the *hpt* gene results in bacteria with a significantly reduced intracellular growth rate and attenuated virulence in mice (14), suggesting that hexose phosphates serve as important carbon sources for growth of *L. monocytogenes* in the cytoplasm.

PrfA HELPS MEDIATE THE *L. MONOCYTOGENES* SWITCH FROM ENVIRONMENTAL DR. JEKYLL TO PATHOGENIC MR. HYDE

Increasing evidence suggests that PrfA is a key part of the potion that transforms Dr. Jekyll into pathogenic Mr. Hyde. Overall, if one were to generate a model (or write a novel) describing the fateful Jekyll-and-Hyde transition of *L. monocytogenes*, it might be best put forward as follows: in response to environmental signals outside of a host, *L. monocytogenes* maintains its Dr. Jekyll persona by repressing both PrfA production and activity through transcriptional (promoter expression), posttranscriptional (RNA thermosensor), and posttranslational mechanisms (PrfA activation), thereby cloaking the expression of its primary virulence factors except for the internalins, which appear to be produced in advance of infection (12, 59). Once the bacteria are ingested by a mammalian host, the increase in temperature and exposure to reduced pH in the stomach stimulates increased production of stress response proteins, internalins, and PrfA, thus beginning the transition to virulence. In the intestine, internalin A mediates attachment and invasion of host epithelial cells with the support of other internalin proteins. Once within the cell phagosome, low iron and low carbohydrate concentrations repress internalin production while PrfA-dependent activation of the P*hly* and P*plcA* promoters allows production of LLO and PlcA to promote lysis of the phagocytic vacuole, thereby enabling entry of the bacteria into the cytosol. Within the cytosol, the full transformation of the *L. monocytogenes* Dr. Jekyll into Mr. Hyde is completed when high levels of active PrfA protein activate transcription from the P*actA* and P*mpl* promoters. The resulting production of ActA and PlcB enables spread of the bacteria to adjacent cells.

While this story in progress features PrfA as the protagonist controlling the *L. monocytogenes* transition from the outside environment to the inside of the host, additional characters are clearly required. Full induction of ActA expression, for example, seems to require additional unknown steps or factors beyond what can be explained by PrfA binding (102, 103). Secondary structure of the 150-bp 5′ untranslated region of the *actA* mRNA has recently been shown to be important in full ActA expression, but the detailed mechanism is as yet unknown (122). Posttranscriptional mechanisms also contribute to synthesis of internalin A and B (110) and LLO (101). Mutations mapping outside of the PrfA locus that affect virulence gene expression in *L. monocytogenes* have been identified (69, 103), suggesting the potential presence of other transcription factors, regulatory elements, and signaling molecules required for the regulation of virulence in *L. monocytogenes*.

THE FINALE: THE GOOD NEWS AND THE BAD NEWS

While the bad news is that *L. monocytogenes* is capable of undergoing the dangerous transition from an environmental Dr. Jekyll to a pathogenic Mr. Hyde within the host, the good news may be that the Mr. Hyde form seems to suffer a competitive disadvantage outside the host. *L. monocytogenes prfA* mutants that contain constitutively activated alleles of *prfA* (and are thus locked into the Mr. Hyde phase) are fully virulent, and in some cases hypervirulent, in mouse models of infection; however, these mutants are severely compromised for flagellum-mediated swimming motility and therefore may be hindered in nutrient acquisition in environments outside the host. It therefore appears that *L. monocytogenes* must maintain a balance between life in the outside environment and life within the host; thus, bacteria that can undergo the switch back to the humble Dr. Jekyll form may be favored over the evolution of increasingly dangerous Mr. Hydes.

The last decade has seen an enormous expansion in our understanding of how *L. monocytogenes* regulates the transition from peaceful saprophyte to deadly pathogen. The switch from environmental microbe to pathogen is mediated by a diverse array of microorganisms encompassing both bacteria and fungi. In addition to *L. monocytogenes*, the organisms able to make the transition from the outside environment to inside a mammalian host include important pathogens such as *Vibrio cholerae* (107), *Bacillus anthracis* (25), *Cryptosporidium parvum* (35), and *L. pneumophila* (79). In most cases there is limited understanding of what molecular mechanisms serve to mediate the switch from life outside the host to life within a host, and, thus, the more we know of the strategies used by one environmental pathogen, *L. monocytogenes*, the better we may understand whether similar strategies might exist and be used by other pathogens to mediate deadly transitions.

ACKNOWLEDGMENTS

L. monocytogenes research in the authors' laboratories is supported by the National Institutes of Health (grants AI41816 and AI055651 to N.E.F. and AI052151 to K.J.B.) and by the Cooperative State Research, Education, and Extension Service, National Research Initiative Competitive Grants Program (NRI Proposal 2005-35201-15330 to K.J.B.) of the U. S. Department of Agriculture.

REFERENCES

1. **Bassler, B. L.** 2002. Small talk. Cell-to-cell communication in bacteria. Cell **109:**421–424.
2. **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.
3. **Becker, L. A., M. S. Çetin, 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.
4. **Begley, M., R. D. Sleator, C. G. M. Gahan, and C. Hill.** 2005. Contribution of three bile-associated loci, *bsh*, *pva*, and *btlB*, to gastrointestinal persistence and bile tolerance of *Listeria monocytogenes*. Infect. Immun. **73:**894–904.
5. **Behari, J., and P. Youngman.** 1998. Regulation of *hly* expression in *Listeria monocytogene*s by carbon sources and pH occurs through separate mechanisms mediated by PrfA. Infect. Immun. **66:**3635–3642.
6. **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.
7. **Bergman, 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. Mol. Microbiol. **43:**557–570.
8. **Böckmann, 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. Mol. Microbiol. **22:**643–653.
9. **Brehm, K., M-.T. Ripio, J. Kreft, and J.-A. Vázquez-Boland.** 1999. The *bvr* locus of *Listeria monocytogenes* mediates virulence gene repression by β-glucosides. J. Bacteriol. **181:**5024–5032.
10. **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. Mol. Gen. Genet. **261:**323–336.
11. **Camilli, A., L. G. Tilney, and D. A. Portnoy.** 1993. Dual roles of *plcA* in *Listeria monocytogenes* pathogenesis. Mol. Microbiol. **8:**143–157.
12. **Chakraborty, T., M. Leimeister-Wächter, 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.
13. **Cheng, L. W., and D. A. Portnoy.** 2003. *Drosophila* S2 cells: an alternative infection model for *Listeria monocytogenes*. Cell Microbiol. **5:**875–885.
14. **Chico-Calero, I., M. Suárez, B. González-Zorn, M. Scortti, J. Slaghuis, W. Goebel, European *Listeria* Genome Consortium, and J. A. Vázquez-Boland.** 2002. Hpt, a bacterial homologue of the microsomal glucose-6-phosphate translocase, mediates rapid intracellular proliferation in *Listeria*. Proc. Natl. Acad. Sci. USA. **99:**431–436.
15. **Christiansen, J. K., M. H. Larsen, H. Ingmer, L. Søgaard-Andersen, and B. H. Kallipolitis.** 2004. The RNA-binding protein Hfq of *Listeria monocytogenes*: role in stress tolerance and virulence. J. Bacteriol. **186:**3355–3362.
16. **Conte, M. P., C. Longhi, M. Polidoro, G. Petrone, V. Buonfiglio, S. Di Santo, E. Papi, L. Seganti, P. Visca, and P. Valenti.** 1996. Iron availability affects entry of *Listeria monocytogenes* into the enterocyte-like cell line Caco-2. Infect. Immun. **64:**3925–3929.
17. **Conte, M. P., C. Longhi, G. Petrone, M. Polidoro, P. Valenti, and L. Seganti.** 2000. Modulation of *actA* expression in *Listeria monocytogenes* by iron. J. Med. Microbiol. **49:**681–683.
18. **Conte, M. P., G. Petrone, A. M. Di Biase, M. G. Ammendolia, F. Superti, and L. Seganti.** 2000. Acid tolerance in *Listeria monocytogenes* influences invasiveness of enterocyte-like cells and macrophage-like cells. Microb. Pathog. **29:**137–144.
19. **Conte, M. P., G. Petrone, A. M. D. Biase, C. Longhi, M. Penta, A. Tinari, F. Superti, G. Fabozzi, P. Visca, and L. Seganti.** 2002. Effect of acid adaptation on the fate of *Listeria monocytogenes* in THP-1 human macrophages activated by gamma interferon. Infect. Immun. **70:**4369–4378.
20. **Cossart, P.** 2002. Molecular and cellular basis of the infection by *Listeria monocytogenes*: an overview. Int. J. Med. Microbiol. **291:**401–409.
21. **Cotter, P. D., N. Emerson, C. G. M. 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.
22. **Cotter, P. D., and C. Hill.** 2003. Surviving the acid test: responses of gram-positive bacteria to low pH. Microbiol. Mol. Biol. Rev. **67:**429–453.
23. **Cowart, R. E., and B. G. Foster.** 1981. The role of iron in the production of haemolysin by *Listeria monocytogenes*. Curr. Microbiol. **6:**287–290.
24. **Dickneite, C., R. Böckmann, A. Spory, W. Goebel, and Z. Sokolovic.** 1998. Differential interaction of the transcription factor PrfA and the PrfA-activating factor (Paf) of *Listeria monocytogenes* with target sequences. Mol. Microbiol. **27:**915–928.
25. **Dixon, T. C., A. A. Fadl, T. M. Koehler, J. A. Swanson, and P. C. Hanna.** 2000. Early *Bacillus anthracis*-macrophage interactions: intracellular survival and escape. Cell Microbiol. **2:**453–463.
26. **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.
27. **Dussurget, O., D. Cabanes, P. Dehoux, M. Lecuit, European *Listeria* Genome Consortium, C. Buchrieser, P. Glaser, and P. Cossart.** 2002. *Listeria monocytogenes* bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. Mol. Microbiol. **45:**1095–1106.
28. **Dussurget, O., J. Pizarro-Cerda, and P. Cossart.** 2004. Molecular determinants of *Listeria monocytogenes* virulence. Annu. Rev. Microbiol. **58:**587–610.
29. **Eiting, M., G. Hagelüken, W.-D. Schubert, and D. W. Heinz.** 2005. The mutation G145S in PrfA, a key virulence regulator of *Listeria monocytogenes*, increases DNA-binding affinity by stabilizing the HTH motif. Mol. Microbiol. **56:**433–446.
30. **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. Mol. Microbiol. **21:**823–837.
31. **Ermolaeva, S., N. Varfolomeeva, Y. Belyi, and I. Tartakovskii.** 1997. Isolation and characterization of a *Listeria monocytogenes* mutant strain hyperproducing virulence factors. FEMS Microbiol. Lett. **150:**189–195.
32. **Ermolaeva, S., Y. Belyi, and I. Tartakovskii.** 1999. Characteristics of virulence factor expression by activated charcoal in *Listeria monocytogenes*. FEMS Microbiol. Lett. **174:**137–141.
33. **Ermolaeva, S., S. Novella, Y. Vega, M.-T. Ripio, M. Scortti, and J. A. Vázquez-Boland.** 2004. Negative control of *Listeria monocytogenes* virulence genes by a diffusible autorepressor. Mol. Microbiol. **52:**601–611.
34. **Falcão, J. P., F. Sharp, and V. Sperandio.** 2004. Cell-to-cell signaling in intestinal pathogens. Curr. Issues Intest. Microbiol. **5:**9–17.
35. **Fayer, R., J. P. Dubey, and D. S. Lindsay.** 2004. Zoonotic protozoa: from land to sea. Trends Parasitol. **20:**531–536.
36. **Fenlon, D. R.** 1999. *Listeria monocytogenes* in the natural environment, p. 21–38. *In* E. T. Ryser and E. H. Marth (ed.), *Listeria*, listeriosis, and food safety, 2nd ed. Marcel Dekker, New York, N.Y.
37. **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.
38. **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.
39. **Fraser, K. R., D. Sue, M. Wiedmann, K. J. Boor, and C. P. O'Byrne.** 2003. Role of σ^B in regulating the compatible solute uptake systems of *Listeria monocytogenes*: osmotic induction of *opuC* is σ^B dependent. Appl. Environ. Microbiol. **69:**2015–2022.
40. **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.
41. **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.
42. **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.
43. **Freitag, N. E., and K. E. Jacobs.** 1999. Examination of *Listeria monocytogenes* intracellular gene expression by using the green fluorescent protein of *Aequorea victoria*. Infect. Immun. **67:**1844–1852.
44. **Gahan, C. G. M., and C. Hill.** 1999. The relationship between acid stress responses and virulence in *Salmonella typhimurium* and *Listeria monocytogenes*. Int. J. Food Microbiol. **50:**93–100.
45. **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.
46. **Garner, M. R., B. L. Njaa, M. Wiedmann, and K. J. Boor.** 2006. Sigma B contributes to *Listeria monocytogenes* gastrointestinal infection but not to systemic spread in the guinea pig infection model. Infect. Immun. **74:**876–886.
47. **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.
48. **Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok, A. Amend, F. Baquero, P. Berche, H. Bloeker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couvé, A. d. Daruvar, P. Dehoux, E. Domann, G. Domínguez-Bernal, E. Duchaud, L. Durant, O. Dussurget, K.-D. Entian, H. Fsihi, F. G.-D. Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gómez-López, T. Hain, J. Hauf, D. Jackson, L.-M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueño, A. Maitournam, J. M. Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. d. Pablos, J.-C. Pérez-Diaz, R. Purcell, B. Remmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J. A. Vázquez-Boland, H. Voss, J. Wehland, and P. Cossart.** 2001. Comparative genomics of *Listeria* species. Science **294:**849–852.
49. **Greub, G., and D. Raoult.** 2004. Microorganisms resistant to free-living amoebae. Clin. Microbiol. Rev. **17:**413–433.
50. **Gruber, T. M., and C. A. Gross.** 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. Annu. Rev. Microbiol. **57:**441–466.
51. **Gründling, A., L. S. Burrack, H. G. A. Bouwer, and D. E. Higgins.** 2004. *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. Proc. Natl. Acad. Sci. USA. **101:**12318–12323.
52. **Hardy, J., K. P. Francis, M. DeBoer, P. Chu, K. Gibbs, C. H. Contag.** 2004. Extracellular replication of *Listeria monocytogenes* in the murine gall bladder. Science **303:**851–853.
53. **Harman, J. G.** 2001. Allosteric regulation of the cAMP receptor protein. Biochim. Biophys. Acta **1547:**1–17.
54. **Herler, M., B. 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.
55. **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:**265–272.
56. **Johansson, J., 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.
57. **Kathariou, S.** 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. **65:**1811–1829.
58. **Kazmierczak, M., S. Mithoe, K. J. Boor, and M. Wiedmann.** 2003. *Listeria monocytogenes* σ^B regulates stress response and virulence functions. J. Bacteriol. **185:**5722–5734.
59. **Kim, H., K. J. Boor, and H. Marquis.** 2004. *Listeria monocytogenes* σ^B contributes to invasion of human intestinal epithelial cells. Infect. Immun. **72:**7374–7378.
60. **Kim, H., H. Marquis, and K. J. Boor.** 2005. σ^B contributes to *Listeria monocytogenes* invasion by controlling expression of *inlA* and *inlB*. Microbiology **151:**3215–3222.
61. **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.
62. **Körner, H., H. J. Sofia, and W. G. Zumft.** 2003. Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. FEMS Microbiol. Rev. **27:**559–592.
63. **Kreft, J., and J. A. Vazquez-Boland.** 2001. Regulation of virulence genes in *Listeria*. Int. J. Med. Microbiol. **291:**145–157.
64. **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.
65. **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. Mol. Microbiol. **13:**141–151.
66. **Lara-Tejero, M., and E. G. Pamer.** 2004. T cell responses to *Listeria monocytogenes*. Curr. Opin. Microbiol. **7:**45–50.
67. **Leimeister-Wächter, M., E. Domann, and T. Chakraborty.** 1992. The expression of virulence genes in *Listeria monocytogenes* is thermoregulated. J. Bacteriol. **174:**947–952.
68. **Litwin, C. M., and S. B. Calderwood.** 1993. Role of iron in regulation of virulence genes. Clin. Microbiol. Rev. **6:**137–149.
69. **Lukowiak, A. M., K. J. Mueller, N. E. Freitag, and P. Youngman.** 2004. Deregulation of *Listeria monocytogenes* virulence gene expression by two distinct and semi-independent pathways. Microbiology **150:**321–333.
70. **Ly, T. M., and H. E. Muller.** 1990. Ingested *Listeria monocytogenes* survive and multiply in protozoa. J. Med. Microbiol. **33:**51–54.
71. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116:** 381–406.
72. **Makino, M., M. Kawai, I. Kawamura, M. Fujita, F. Gejo, and M. Mitsuyama.** 2005. Involvement of reactive oxygen intermediate in the enhanced expression of virulence-associated genes of *Listeria monocytogenes* inside activated macrophages. Microbiol. Immunol. **49:**805–811.
73. **Mansfield, B. E., M. S. Dionne, D. S. Schneider, and N. E. Freitag.** 2003. Exploration of host-pathogen interactions using *Listeria monocytogenes* and *Drosophila melanogaster*. Cell Microbiol. **5:**901–911.
74. **Marron, L., N. Emerson, C. G. M. Gahan, and C. Hill.** 1997. A mutant of *Listeria monocytogenes* LO28 unable to induce an acid tolerance response displays diminished virulence in a murine model. Appl. Environ. Microbiol. **63:**4945–4947.
75. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCraig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
76. **Mengaud, J., S. Dramsi, E. Gouin, J. A. Vázquez-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.
77. **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.
78. **Milohanic, E., P. Glaser, J.-Y. Coppée, L. Frangeul, Y. Vega, J. A. Vázquez-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.
79. **Molofsky, A. B., and M. S. Swanson.** 2004. Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. Mol. Microbiol. **53:**29–40.
80. **Moors, M. A., B. Levitt, P. Youngman, and D. A. Portnoy.** 1999. Expression of listerolysin O and ActA by intracellular and extracellular *Listeria monocytogenes*. Infect. Immun. **67:**131–139.
81. **Moran, C. P.** 1993. RNA polymerase and transcription factors, p. 653–667. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, D.C.
82. **Morita, M. T., Y. Tanaka, T. S. Kodama, Y. Kyogoku, H. Yanagi, and T. Yura.** 1999. Translational induction of heat shock transcription factor σ^{32}: evidence for a built-in RNA thermosensor. Gene Dev. **13:**655–665.
83. **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.
84. **Nelson, K. E., D. E. Fouts, E. F. Mongodin, J. Ravel, R. T. DeBoy, J. F. Kolonay, D. A. Rasko, S. V. Angiuoli, S. R. Gill, I. T. Paulsen, J. Peterson, O. White, W. C. Nelson, W. Nierman, M. J. Beanan, L. M. Brinkac, S. C. Daugherty, R. J. Dodson, A. S. Durkin, R. Madupu, D. H. Haft, J. Selengut, S. Van Aken, H. Khouri, N. Fedorova, H. Forberger, B. Tran, S. Kathariou, L. D. Wonderling, G. A. Uhlich, D. O. Bayles, J. B. Luchansky, and C. M. Fraser.** 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. Nucleic Acids Res. **32:**2386–2395.
85. **O'Driscoll, B., C. G. M. Gahan, and C. Hill.** 1996. Adaptive acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. Appl. Environ. Microbiol. **62:**1693–1698.
86. **Pamer, E. G.** 2004. Immune responses to *Listeria monocytogenes*. Nat. Rev. Immunol. **4:**812–823.
87. **Peel, M., W. Donachie, and A. Shaw.** 1988. Temperature-dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and western blotting. J. Gen. Microbiol. **134:**2171–2178.
88. **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.
89. **Portnoy, D. A., P. S. Jacks, and D. Hinrichs.** 1988. Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. J. Exp. Med. **167:**1459–1471.
90. **Portnoy, D. A., T. Chakraborty, W. Goebel, and P. Cossart.** 1992. Molecular determinants of *Listeria monocytogenes* pathogenesis. Infect. Immun. **60:** 1263–1267.
91. **Rauch, M., Q. Luo, S. Muller-Altrock, and W. Goebel.** 2005. σ^B-Dependent in vitro transcription of *prfA* and some newly identified genes of *Listeria monocytogenes* whose expression is affected by PrfA in vivo. J. Bacteriol. **187:**800–804.
92. **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.
93. **Ripio, M.-T., G. Domíngez-Bernal, M. Suárez, K. Brehm, P. Berche, and J.-A. Vázquez-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.
94. **Ripio, M.-T., K. Brehm, M. Lara, M. Suárez, and J.-A. Vázquez-Boland.** 1997. Glucose-1-phosphate utilization by *Listeria monocytogenes* is PrfA dependent and coordinately expressed with virulence factors. J. Bacteriol. **179:**7174–7180.
95. **Ripio, M.-T., G. Domínguez-Bernal, M. Lara, M. Suárez, and J.-A. Vázquez-Boland.** 1997. A Gly145Ser substitution in the transcriptional activator PrfA causes constitutive overexpression of virulence factors in *Listeria monocytogenes*. J. Bacteriol. **179:**1533–1540.
96. **Roberts, A. J., and M. Wiedmann.** 2003. Pathogen, host, and environmental factors contributing to the pathogenesis of listeriosis. Cell Mol. Life Sci. **60:**1–15.
97. **Saklani-Jusforgues, H., E. Fontan, and P. L. Goossens.** 2000. Effect of acid-adaptation on *Listeria monocytogenes* survival and translocation in a murine intragastric infection model. FEMS Microbiol. Lett. **193:**155–159.
98. **Schwab, U., B. Bowen, C. Nadon, M. Wiedmann, and K. J. Boor.** 2005. The *Listeria monocytogenes prfA*P2 promoter is regulated by σ^B in a growth phase dependent manner. FEMS Microbiol. Lett. **245:**329–336.
99. **Seeliger, H. P. R., and D. Jones.** 1986. Genus *Listeria*, p. 1235–1245. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. Williams & Wilkins, Baltimore, Md.
100. **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.
101. **Shen, A., and D. E. Higgins.** 2005. The 5′ untranslated region-mediated enhancement of intracellular listeriolysin O production is required for *Listeria monocytogenes* pathogenicity. Mol. Microbiol. **57:**1460–1473.
102. **Shetron-Rama, L. M., H. Marquis, H. G. A. Bouwer, and N. E. Freitag.** 2002. Intracellular induction of *Listeria monocytogenes actA* expression. Infect. Immun. **70:**1087–1096.
103. **Shetron-Rama, L. M., K. Mueller, J. M. Bravo, H. G. A. Bouwer, S. S. Way, and N. E. Freitag.** 2003. Isolation of *Listeria monocytogenes* mutants with high-level in vitro expression of host cytosol-induced gene products. Mol. Microbiol. **48:**1537–1551.
104. **Sleator, R. D., H. H. Wemekamp-Kamphuis, C. G. M. Gahan, T. Abee, and C. Hill.** 2005. A PrfA-regulated bile exclusion system (BilE) is a novel virulence factor in *Listeria monocytogenes*. Mol. Microbiol. **55:**1183–1195.
105. **Smith, G. A., H. Marquis, S. Jones, N. C. Johnston, D. A. Portnoy, and H. Goldfine.** 1995. The two distinct phospholipases C of *Listeria monocytogenes* have overlapping roles in escape from a vacuole and cell-to-cell spread. Infect. Immun. **63:**4231–4237.
106. **Sokolovic, Z., J. Riedel, M. Wuenscher, and W. Goebel.** 1993. Surface-associated, PrfA-regulated proteins of *Listeria monocytogenes* synthesized under stress conditions. Mol. Microbiol. **8:**219–227.

107. **Soomro, A. L., and N. Junejo.** 2004. *Vibrio cholerae* in the environment. J. Coll. Physicians Surg. Pak. **14:**509–512.
108. **Stevenson, R. L.** 1886. The strange case of Dr. Jekyll and Mr. Hyde. Scribner, New York, N.Y.
109. **Straus, D. B., W. A. Walter, and C. A. Gross.** 1987. The heat shock response of *E. coli* is regulated by changes in the concentration of σ^{32}. Nature **329:**348–351.
110. **Stritzker, J., C. Schoen, and W. Goebel.** 2005. Enhanced synthesis of internalin A in *aro* mutants of *Listeria monocytogenes* indicates posttranscriptional control of the *inlAB* mRNA. J. Bacteriol. **187:**2836–2845.
111. **Swanson, M. S., and B. K. Hammer.** 2000. *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages. Annu. Rev. Microbiol. **54:**567–613.
112. **Thirumuruhan, R., K. Rajashankar, A. A. Fedorov, T. Dodatko, M. R. Chance, P. Cossart, and S. C. Almo.** 11 March 2003, posting date. Crystal structure of PrfA, the transcriptional regulator in *Listeria monocytogenes*. www.rcsb.org/pdb/cgi/explore.cgi?pdbId=1OMI. [Online.]
113. **Tilney, L. G., and D. A. Portnoy.** 1989. Actin filaments and the growth, movement, and spread of the intracellular parasite, *Listeria monocytogenes*. J. Cell Bio. **109:**1597–1608.
114. **Vázquez-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.
115. **Vázquez-Boland, J.-A., M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland, and J. Kreft.** 2001. *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. **14:**584–640.
116. **Vega, Y., M. Rauch, M. J. Banfield, S. Ermolaeva, M. Scortti, W. Goebel, and J. A. Vázquez-Boland.** 2004. New *Listeria monocytogenes prfA** mutants, transcriptional properties of PrfA* proteins and structure-function of the virulence regulator PrfA. Mol. Microbiol. **52:**1553–1565.
117. **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.
118. **Wemekamp-Kamphuis, H. H., J. A. Wouters, P. P. L. A. de Leeuw, T. Hain, T. Chakraborty, and T. Abee.** 2004. Identification of sigma factor σ^B-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria monocytogenes* EGD-e. Appl. Environ. Microbiol. **70:**3457–3466.
119. **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.
120. **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.
121. **Wong, K. K. Y., and N. E. Freitag.** 2004. A novel mutation within the central *Listeria monocytogenes* regulator PrfA that results in constitutive expression of virulence gene products. J. Bacteriol. **186:**6265–6276.
122. **Wong, K. K. Y., H. G. A. Bouwer, and N. E. Freitag.** 2004. Evidence implicating the 5′ untranslated region of *Listeria monocytogenes actA* in the regulation of bacterial actin-based motility. Cell Microbiol. **6:**155–166.

Editor: J. B. Kaper