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Modulation of stress and virulence in *Listeria monocytogenes*

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Abstract

Listeria monocytogenes can respond rapidly to changing environmental conditions, as illustrated by its ability to transition from a saprophyte to an orally transmitted facultative intracellular pathogen. Differential associations between various alternative σ factors and a core RNA polymerase provide a transcriptional mechanism for regulating bacterial gene expression that is crucial for survival in rapidly changing conditions. Alternative σ factors are key components of complex *L. monocytogenes* regulatory networks that include multiple transcriptional regulators of stress-response and virulence genes, regulation of genes encoding other regulators, and regulation of small RNAs. In this article, the contributions of various σ factors to *L. monocytogenes* stress response and virulence are described.

Listeria monocytogenes – a facultative intracellular pathogen and an environmental saprophyte

The Gram-positive bacterium *Listeria monocytogenes* is described as having a Jekyll and Hyde lifestyle [1] because it can cause life-threatening invasive disease in humans and animals but can also effectively survive and multiply in many different non-host environments. In fact, the robust ability of *L. monocytogenes* to survive and multiply outside a host is crucial to its transmission, which typically occurs via the consumption of contaminated foods or animal feeds. *L. monocytogenes* has several regulatory mechanisms that contribute to its ability to respond to and survive under rapidly changing conditions. Regulatory mechanisms contributing to bacterial survival can function at various levels, including the transcriptional level, the post-transcriptional level and the post-translational level. This review focuses specifically on the contributions of alternative σ factors to transcriptional regulation in *L. monocytogenes*, including the contributions of these regulatory mechanisms to stress resistance, environmental survival and virulence.

The pathogenic lifestyle of L. monocytogenes

L. monocytogenes can cause disease (listeriosis) in several animal species. It predominantly occurs in mammals but is also seen in some poikilothermic animals and avian species [2]. Although *L. monocytogenes* can cause mild illness (e.g. diarrhea and flu-like symptoms) in humans, it also can cause severe invasive infections. Listeriosis manifestations in humans include meningitis, encephalitis and septicemia, and spontaneous abortions and stillbirths or infant septicemia and meningitis after the infection of pregnant women [3]. The majority (99%) of human listeriosis infections are foodborne [4]. Because initial contamination of

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food products usually occurs at low levels and the human infectious dose seems to be high [5], post-contamination multiplication of *L. monocytogenes* in food products usually needs to occur for the pathogen to reach high enough levels to cause human disease. Thus, the transmission of infectious doses of *L. monocytogenes* to human hosts is often dependent on the ability of this pathogen to survive and multiply in diverse environments, including in refrigerated foods.

After the consumption of contaminated foods, human listeriosis infections involve the following steps: (i) bacterial survival through the gastric passage; (ii) survival and colonization in the intestinal tract, with possible intestinal symptoms (diarrhea); (iii) invasion of intestinal epithelial cells and/or microfold (M) cells [6]; and (iv) systemic spread with intracellular infection of phagocytic and non-phagocytic cells. Cellular-infection stages also have been defined as follows: (i) internalization of *L. monocytogenes* within the host cell; (ii) bacterial escape from the host vacuole; (iii) bacterial multiplication within the host-cell cytoplasm and movement through the host cytoplasm by bacterially directed nucleation of host actin; (iv) bacterial movement to the host-cell surface and extrusion of bacterial cells in pseudopod-like structures; and (v) phagocytosis of these pseudopod-like structures by neighboring cells, followed by escape of the bacterium from the resulting double-membrane vacuole. The escape of the bacterium enables repetition of the cycle. Several *L. monocytogenes* virulence genes and their specific functions during these stages of intracellular infection have been identified and characterized, including *prfA*, which encodes positive regulatory factor A (PrfA) [5] (Box 1).

Box 1

Listeria monocytogenes stress-response and virulence genes and proteins, and abbreviations

Transcription regulators

CodY: transcription repressor that responds to GTP and branched-chain amino acid levels in the cell.

CtsR: class III stress-response regulator; a transcription repressor.

Hfq: RNA-binding protein involved in stress resistance and virulence.

HrcA: heat regulation at controlling inverted-repeat of chaperone expression (CIRCE) elements; a transcription repressor.

PrfA: positive regulatory factor A; global regulator that controls transcription of many *L. monocytogenes* genes that are essential for virulence.

Genes involved in virulence

bilE **(formerly** ***lmo1421-lmo1422*****, or** ***opuBAB*****)**: this two-gene operon (*bilEAB*) encodes a bile-exclusion system; responsible for bile resistance.

bsh: encodes bile salt hydrolase, which is important for bile tolerance.

clpC **operon**: comprises *ctsR, mcsA*, *mcsB* and *clpC*, encoding CtsR, McsA and McsB, which both regulate CtsR activity, and the ClpC endopeptidase and chaperone.

inlAB: this operon encodes internalin A and internalin B.

LIPI: *Listeria* pathogenicity island; comprises virulence genes *prfA, plcA, hly, mpl, actA* and *plcB.*

Genes involved in general-stress response

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Acid stress

GAD: the glutamate decarboxylase system, which is encoded by the *gad* genes. *gadA* (formerly *gadB, gadD, gadD3* or *lmo2434*) encodes glutamate decarboxylase. The *gadCB* (formerly *gadD2T2* or *lmo2363–lmo2362*) operon encodes a glutamate decarboxylase and a glutamate-γ-aminobutyric acid (GABA) antiporter.

Osmotic stress

BetL: glycine betaine transporter encoded by *betL*.

Gbu: glycine betaine ABC transport system encoded by a three-gene operon (*gbuABC*).

OpuC: carnitine ATP-binding cassette (ABC) transport system encoded by a four-gene operon (*opuCABCD*).

Oxidative stress

lmo0669: encodes an oxidoreductase; transcribed in an operon with *lmo0670,* which encodes a hypothetical protein.

lmo1433: encodes glutathione reductase.

Energy stress and cellular metabolism

ldh: encodes L-lactate dehydrogenase.

lmo0398–lmo0400: operon encodes a fructose-specific phospho-transferase system (PTS).

lmo0784–lmo0781: operon encodes a mannose-specific PTS.

lmo2205: encodes phosphoglyceromutase.

lmo2460: encodes a transcriptional regulator similar to the central glycolytic gene regulator (CggR).

pdhB: encodes pyruvate dehydrogenase (E1 β subunit).

Cold stress

cspD: encodes cold-shock protein *D*.

ltrC: encodes low-temperature requirement C protein.

Other genes or proteins

clpB: encodes ClpB, similar to endopeptidase Clp ATP-binding chain B; transcribed in an operon with *lmo2205*.

clpP: encodes ClpP, an ATP-dependent Clp protease proteolytic subunit.

clpX: encodes ClpX, an ATP-dependent Clp protease ATP-binding subunit.

DnaKJ: heat-shock proteins encoded by the *dnaK* operon consisting of *hrcA, grpE, dnaK, dnaJ, lmo1471* and *lmo1470*.

groESL: encode chaperone proteins GroES and GroEL, which regulate HrcA post-transcriptionally.

grpE: encodes heat-shock protein GrpE.

lmo1138: similar to ATP-dependent Clp protease proteolytic component.

lmo1601: encodes a general-stress protein; transcribed in an operon with *lmo1602*, which encodes an unknown protein.

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lmo1602: encodes an unknown protein; transcribed in an operon with *lmo1601*.

lmo2230: encodes a protein similar to arsenate reductase.

lstR: encodes lineage-specific thermal regulator; transcribed in an operon with *sigC*.

rsbVWX: encode regulators of σ^B activity, RsbV, RsbW and RsbX.

Other abbreviations

CCCP: carbonyl cyanide 3-chloropenylhydrazone. A protonophore used to destroy the proton motive force across a plasma membrane to cause inhibition of ATP synthesis and depletion of cellular energy in a bacterial cell.

CHP: cumene hydroperoxide. A compound used to induce oxidative stress in the cell.

ppGpp: Guanosine tetraphosphate. An effector of the stringent response and a regulator of differential binding of alternative σ factors to RNA polymerase.

The nonpathogenic lifestyle of L. monocytogenes

L. monocytogenes is ubiquitously present in the environment. It has been isolated from several non-host-associated locations, including a variety of raw and ready-to-eat human foods, food-processing plants, retail food operations, urban environments, farm environments and natural environments that have had limited human impact [7]. Characterization of *L. monocytogenes* isolates from various hosts and environments by using a variety of different subtyping methods indicates that strains comprising the species *L. monocytogenes* represent at least three distinct genetic lineages [7]. Serotyping is a method that is commonly used to characterize *L. monocytogenes* strains. Thirteen *L. monocytogenes* serotypes have been differentiated; three serotypes (1/2a, 1/2b and 4b) represent the vast majority of human listeriosis isolates [3].

L. monocytogenes is capable of reproducing to high numbers in different natural environments. For example, naturally contaminated silage on farms has been reported to carry 1×10^8 colony-forming units (CFU) per g [8] and naturally contaminated human foods can contain $>10^3$ CFU per g [9], although the majority of food samples tested and reported in Ref. [9] contained <1 CFU per g. These findings clearly illustrate the ability of *L. monocytogenes* to compete and grow successfully in non-host environments.

Under laboratory conditions, *L. monocytogenes* can grow in a wide range of environmental conditions, including temperatures of +1 °C–45 °C, a pH range of 4.4–9.6 and salt concentrations up to 10% weight/volume NaCl [10]. *L. monocytogenes* can persist, although it might not grow, under an even wider range of conditions, including pH levels as low as 2.5 [11], bile and bile-acid concentrations higher than the 0.3% volume/volume and 5 mM, respectively, that are typically encountered in host animals [12], and high hydrostatic pressures [13]. Moreover, in addition to surviving under specific stress conditions, exposing *L. monocytogenes* to mild conditions of one type of stress can produce cross-protection against other stresses and result in robust and persistent strains in a given environment [14,15].

L. monocytogenes transcription regulation in external and intrahost environments

One important mechanism for regulating the transcription of appropriate genes under rapidly changing environmental conditions is the ability of different alternative σ factors to associate with a core RNA polymerase under specific environmental conditions. The σ

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factor is a dissociable subunit of the RNA polymerase holoenzyme that is responsible for enzyme recognition of a specific DNA sequence that represents a promoter region for a given gene and for transcription initiation at that promoter site. Therefore, differential associations between alternative σ factors and core polymerase, in essence, reprogram promoter-recognition specificities of the enzyme, thus enabling expression of new sets of target genes under different environmental conditions. Some alternative σ factors (e.g. σ^B) are maintained in inactive states through direct interactions between a given σ factor and its specific anti-σ-factor protein until the cell encounters environmental conditions requiring expression of genes recognized by that σ factor (as reviewed in Ref. [16]). Guanosine tetraphosphate (ppGpp) seems to have an important role in σ-factor competition for core RNA polymerase [17,18]). Alternative σ factors can produce dramatic changes in bacterial gene expression after exposure to environmental stress conditions, such as during the sporulation process of *Bacillus subtilis*, which is controlled by association of a specific cascade of alternative σ factors with core polymerase [19]. The *L. monocytogenes* genome encodes four alternative σ factors (σ^B, σ^C, σ^H and σ^L) [20]. By contrast, the genome of the human intestinal bacterium *Bacteroides thetaiotaomicron* encodes 50 alternative σ factors [21] and *B. subtilis* encodes 18 [22].

L. monocytogenes *alternative* σ factor σ^B

σ^B is encoded by *sigB*. Identification of the *L. monocytogenes* σ^B regulon, combined with proteomic [23] and phenotypic analyses of *sigB*-null mutants (Δ*sigB*), has provided considerable evidence that σ^B crucially contributes to the ability of *L. monocytogenes* to multiply and survive, both in mammalian hosts and under stress conditions encountered in non-host-associated environments [1]. σ^B was first identified and characterized in *B. subtilis* and subsequently has been identified in several other Gram-positive bacteria including *L. monocytogenes*, *Staphylococcus aureus*, *Bacillus anthracis* and *Bacillus licheniformis* [16]. Characterization of the *L. monocytogenes* σ^B regulon during stationary phase and under salt stress identified >170 positively regulated σ^B-dependent genes in *L. monocytogenes*, including 145 genes that are preceded by a putative σ^B-dependent promoter, indicating that they are directly regulated by σ^B [24,25]. The *L. monocytogenes* σ^B regulon seems to be similar in size to that of *B. subtilis* (125–150 genes [26,27]) and *S. aureus* (198 genes [28]).

Role of σ^B in L. monocytogenes' general-stress response

Both gene-expression and phenotypic data support contributions of σ^B to *L. monocytogenes* response and survival under non-host-associated environmental stress conditions, including acid stress, osmotic stress, oxidative stress, cold stress and nutrient limitation or energy stress. Based solely on gene-expression profiles, σ^B also might be involved in arsenate resistance [24,25] (Table 1).

σ^B clearly contributes to *L. monocytogenes* acid resistance. Specifically, phenotypic experiments have shown that an *L. monocytogenes* Δ*sigB* mutant has a severely reduced ability to survive acidic conditions (pH 2.5) compared with its parent strain [29,30]. σ^B regulates the transcription of genes contributing to acid resistance, including genes encoding components of the glutamate decarboxylase (GAD) system. In general, the GAD system consists of a glutamate decarboxylase enzyme (e.g. *gadD2*) and a glutamate-γ-aminobutyrate (GABA) antiporter (e.g. *gadT2*) that act in concert to reduce acidification within the cytoplasm of the cell. *L. monocytogenes lmo2434* (also referred to as *gadA* [14,25,31], *gadB* [24], gadD [29] or *gadD3* [32]) seems to be directly regulated by σ^B [24,25,29,31]. In addition, the higher transcript levels observed in Δ*sigB* relative to the parent strain for the *lmo2363–lmo2362* operon (also called the *gadCB* [14] or the *gadD2T2* operons [32]) indicate that these genes are indirectly regulated by σ^B [25].

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σ^B contributes to the osmotic stress-resistance mechanisms of *L. monocytogenes*; σ^B activity increases with increased medium osmolarity [33,34]. The ability of *L. monocytogenes* to grow under conditions of high osmolarity is affected by its ability to accumulate compatible solutes by transport systems. Three σ^B-dependent systems (OpuC, Gbu and BetL) transport carnitine and glycine betaine across the bacterial membrane, from the environment into the cytoplasm, to combat osmotic stress [35,36]. An *L. monocytogenes* Δ*sigB* mutant (10403S, serotype 1/2a) is deficient in its ability to use carnitine and betaine as osmoprotectants compared with the corresponding wild-type strain [33]; however, this phenotype is not shared with a serotype 4c Δ*sigB* mutant [37]. Microarray analyses have revealed that several other transporters and transport systems are upregulated by σ^B in an *L. monocytogenes* 10403S parent strain relative to a Δ*sigB* mutant upon exposure of logarithmic-phase cells to 0.5 M KCl [24] or 0.3 M NaCl [25]. Furthermore, proteomic and reverse-transcription (RT)-PCR analyses showed σ^B-dependent expression of several proteins and genes in the presence of 0.5 M NaCl [23].

Transcriptional profiling of the σ^B regulon revealed several σ^B-dependent genes that encode proteins with putative roles in oxidative-stress resistance, including *lmo0669* and *lmo1433* [24,25,38]. Survival experiments with stationary-phase *L. monocytogenes* 10403S (serotype 1/2a) and its isogenic Δ*sigB* mutant during exposure to ~13 mM cumene hydroperoxide (CHP) showed considerably reduced survival of the Δ*sigB* strain relative to the parent strain and indicated a role for σ^B in oxidative-stress resistance in 10403S [30,39]. However, σ^B contributions to oxidative-stress survival seem to vary among *L. monocytogenes* strains of different serotypes. CHP experiments with a different serotype 1/2a parent strain (*L. monocytogenes* L61) and its isogenic Δ*sigB* mutant (Δ*sigB*61) showed no statistical difference in survival [37]. Interestingly, an *L. monocytogenes* serotype 4c strain (L99) was significantly less resistant than its corresponding Δ*sigB* mutant (Δ*sigB*99) upon exposure to CHP for 15 min [37]. These phenotypic experiments indicate considerable strain-to-strain variability in σ^B contributions to oxidative-stress resistance in *L. monocytogenes*.

Whole-genome transcriptional profiling of *L. monocytogenes* clearly indicated a major role for σ^B in regulating carbohydrate metabolism; identification of numerous σ^B-dependent genes that were upregulated during stationary phase indicates that σ^B is important for utilizing the limited nutrients present in the environment [25,38]. Several operons contributing to carbohydrate metabolism are σ^B-dependent, including *lmo0784–lmo0781* and *lmo0398–lmo0400*, which encode mannose-specific and fructose-specific phosphotransferase systems, respectively [25]. In addition, the presence of a putative σ^B-dependent promoter upstream of *lmo2460*, which encodes a transcriptional regulator similar to the central glycolytic gene regulator (CggR) in *B. subtilis*, indicates that σ^B has a direct role in regulating genes involved in glycolysis and gluconeogenesis [25]. In phenotypic experiments, Δ*sigB* cell numbers declined rapidly compared to those of the *L. monocytogenes* 10403S parent strain during static incubation in brain heart infusion (BHI) broth at 37 °C for 36 h [30]. Enhanced survival of the 10403S parent relative to the Δ*sigB* strain under conditions of energy stress generated by limiting ATP synthesis through addition of the protonophore carbonyl cyanide 3-chloropenylhydrazone (CCCP) to the growth medium indicated that σ^B contributes to energy-stress survival [30]. Quantitative RT-PCR on RNA isolated from *L. monocytogenes* 10403S and otherwise isogenic Δ*sigB* cells exposed to CCCP for five minutes showed that RNA transcript levels for the σ^B-dependent *opuCA* were significantly higher in the parent strain than in the Δ*sigB* mutant, further indicating activation of σ^B in response to CCCP [40]. However, σ^B contributions to energy and carbon limitation seem to be strain dependent. Whereas the *L. monocytogenes* 10403S (serotype 1/2a) response to carbon limitation was σ^B-dependent [30,39], the long-term viability of *L. monocytogenes* L61 (serotype 1/2a) and L99 (serotype 4c) strains under carbon-starvation conditions seemed to be σ^B-independent [37].

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Role of σ^B in L. monocytogenes virulence

Multiple lines of evidence indicate that σ^B contributes to *L. monocytogenes* virulence [16]. For example, σ^B directly regulates expression of some virulence genes and stress-response systems that are required for survival and multiplication under conditions that are encountered in a host, either during gastrointestinal passage (e.g. acidic pH in the gastric environment) or during intracellular survival and growth (e.g. oxidative stress in the host-cell phagosome).

Microarray analyses and single-gene transcriptional analyses have identified several virulence genes that are directly regulated by *L. monocytogenes* σ^B. Specifically, σ^B seems to contribute to the transcriptional regulation of genes important for bile resistance, including *bsh*, which encodes a bile-salt hydrolase [41], and the *bilE (opuB)* operon, which encodes a bile-exclusion system [42]. σ^B also regulates transcription of several genes that encode *L. monocytogenes* internalins, including *inlAB* that encode internalin A and B, which are crucial for *L. monocytogenes* attachment to and invasion of different host-cell types [24,43]. Importantly, σ^B also contributes to transcription of the gene encoding the global *L. monocytogenes* virulence gene regulator, PrfA [44,45] (Box 2).

Box 2

Regulation of PrfA expression by σ^B

σ^B activates transcription at the *prfA*P2 promoter region, which includes overlapping σ^B- and σ^A-dependent promoters, and represents one of three promoter regions that contribute to *prfA* transcription. The other *prfA* promoters are the P1 promoter (which is located upstream of *prfA*P2) and the *plcA* promoter (which can generate a bicistronic transcript that includes *plc*A and *prfA*). Importantly, although translation of the *prfA*P1 transcript is temperature dependent (i.e. translated only at temperatures >30 °C), transcripts originating from the *prfA*P2 promoter do not show temperature-dependent translation [78]. Thus, transcription originating from *prfA*P2 provides a mechanism for σ^B-dependent production of PrfA, even at temperatures typically encountered outside a mammalian host. Inactivation of σ^B-dependent *prfA* transcription through deletion of the *prfA*P2 promoter does not affect *L. monocytogenes* virulence in intragastrically inoculated guinea pigs [50] or *L. monocytogenes*' cell-to-cell spread in at least some mammalian cell lines [79]. The contribution of σ^B to *prfA* transcription thus seems to be important only at specific stages of infection and/or in specific hosts. Alternatively, σ^B transcription at *prfA*P2 might represent a redundant system that provides highly robust regulation of *prfA* expression. Interestingly, the role of σ^B in regulating a primary virulence gene regulator is not unique to *L. monocytogenes*. In *S. aureus*, σ^B contributes to regulation of virulence gene expression through transcriptional regulation of *sar* [28,80]. The *sar* locus partially controls expression of the *agr* locus; *agr* and *sar* are both global regulatory elements that control the synthesis of a variety of extracellular and cell-surface proteins involved in the pathogenesis of *S. aureus* [81].

In addition to regulation of virulence-gene expression (e.g. *prfA*, *bsh* and *inlAB*), *L. monocytogenes* σ^B also regulates transcription of stress-response genes that contribute to the ability of *L. monocytogenes* to infect mammalian hosts. For example, σ^B regulates the *opuC* operon, which encodes an ATP-binding cassette (ABC) transporter of osmoprotectant molecules (predominantly carnitine) that contribute to *L. monocytogenes* survival in the gastrointestinal tract of experimentally infected mice [46]. Another example of σ^B–dependent gene expression that contributes to bacterial survival is induction of glutamate decarboxylase (*gad*) genes when *L. monocytogenes* is exposed to mildly acidic conditions (pH 5.1) [32]. Furthermore, *L. monocytogenes* acid adaptation results in cross-protection

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against other cellular stresses [15], and acid-adapted *L. monocytogenes* also grows markedly better in interferon-γ (IFN-γ)-activated THP-1 macrophages than cultures not previously exposed to acid [14]. σ^B also seems to regulate expression of *L. monocytogenes* genes after entry into both phagocytic and non-phagocytic host cells [47,48]. Among the σ^B-dependent genes differentially transcribed in infected host cells, some genes seem to be predominantly regulated by σ^B (e.g. *lmo1433*, which encodes glutathione reductase) [25,47,48], whereas other genes seem to be co-regulated by σ^B and one or more transcriptional regulators, including PrfA [42,47].

Phenotypic evidence also supports important σ^B-mediated contributions to virulence. For example, σ^B clearly contributes to *L. monocytogenes* survival in artificial gastric fluid with a pH of 2.5 [39]. A Δ*sigB* strain was defective relative to the 10403S parent strain in invading Caco-2 and HepG-2 cells [49], and in causing infections in guinea pigs [50]. σ^B seems to contribute to virulence across multiple Gram-positive bacterial pathogens. For example, a *B. anthracis* Δ*sigB* strain was less virulent than its parent strain, indicating σ^B-mediated virulence contributions in this pathogen [51].

Gene regulation in *L. monocytogenes* by the alternative σ factor σ^H

L. monocytogenes σ^H, encoded by *sigH*, shows homology to the *B. subtilis* stationary-phase σ factor, σ^H, which regulates >400 genes (~240 genes positively and ~180 negatively) [52]. *B. subtilis* σ^H is involved in the transition from exponential phase to stationary phase, sporulation, nutrient transport, and the regulation of many other transcription factors and cell-wall-binding proteins. Although σ^H-dependent *L. monocytogenes* genes have not been identified, an *L. monocytogenes sigH*-null mutant (Δ*sigH*) showed reduced growth in a minimal medium and in alkaline conditions compared with wild type [53]. In addition, characterization of an *L. monocytogenes* Δ*sigH* mutant in an intraperitoneal inoculated mouse model also indicated a role for σ^H in infection [53]. In the closely related pathogen *S. aureus*, σ^H contributes to the expression of competence-related genes (e.g. *comE* and *comG*), indicating a possible indirect role for σ^H in *S. aureus* virulence, by enabling the acquisition of genetic material that is beneficial to bacterial survival and infection [54]. In *B. anthracis*, σ^H contributes to the expression of AtxA, a positive regulator of anthrax toxin components, which indicates a direct role for σ^H in *B. anthracis* virulence [55].

Gene regulation in *L. monocytogenes* by the alternative σ factor σ^L

L. monocytogenes σ^L (also known as σ^{54}, or RpoN), which is closely related to the alternative σ factor σ^{54} in several other bacterial species [56], is encoded by *sigL*. *sigL* expression is sensitive to growth phase and temperature [57,58]. For example, *sigL* expression is induced in logarithmic-phase cells grown at 10 °C [57]; however, it is downregulated during stationary phase at 4 °C [58]. σ^L-dependent transcription requires energy to melt target promoter sequences; therefore, transcription initiation typically requires an additional activator (i.e. ManR) that is also an ATP–GTP-binding protein [59]. Global transcriptome analysis of an *L. monocytogenes rpoN*-null mutant (Δ*sigL*) identified 77 σ^L-dependent genes, including several genes that contribute to carbohydrate and amino acid metabolism [60]. Twenty-four σ^L-dependent genes are differentially expressed inside macrophage cells relative to their expression levels in BHI [47]. Genes encoding for pyruvate dehydrogenase, cell division and thioredoxin reductase are repressed, and those encoding for an oligo-peptide transporter and the ClpP serine protease are induced. These findings indicate roles for σ^L in intracellular replication and stress response. Phenotypic characterization of Δ*sigL* mutants has shown that σ^L contributes to *L. monocytogenes* osmotolerance [61] and influences susceptibility to the antibacterial peptide mesentericin Y105 [56].

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Gene regulation in *L. monocytogenes* by the alternative σ factor σ^C

Extracytoplasmic function (ECF) σ factors comprise a distinct subgroup among σ factors. ECF σ factors typically regulate gene expression in response to envelope or extra-cytoplasmic stimuli, transport and secretion. The first recognized ECF σ factor (encoded by *rpoE*) was identified in *Escherichia coli* [62,63]. Seven ECF σ factors have been identified in *B. subtilis* [22]. Typically, although not in all cases, ECF σ factors are cotranscribed with corresponding anti-σ factors. *L. monocytogenes* encodes only a single ECF σ factor (σ^C). To date, *sigC*, which encodes σ^C, seems to be present only in strains classified into one *L. monocytogenes* evolutionary lineage (lineage II) [64]. *L. monocytogenes* σ^C seems to be activated upon heat stress, and a Δ*sigC* strain showed increased sensitivity to heat killing [64]. No virulence or virulence-associated phenotypes have been ascribed to *L. monocytogenes* σ^C. Expression of *sigC* and of *lstR*, which is immediately downstream of *sigC*, is repressed by the pleiotropic transcriptional regulator CodY during exponential growth, indicating interactions among these transcriptional regulators, and a possible role for CodY in mediating temperature stress (i.e. repressing thermal-resistance genes and cold-stress-response genes) [65].

L. monocytogenes regulatory networks and interactions involving alternative σ factors

L. monocytogenes alternative σ factors clearly have crucial roles in assuring appropriate gene expression during survival of non-host associated stress conditions and during infection. Emerging evidence indicates that alternative σ factors are crucial components of regulatory networks in *L. monocytogenes* and function to (i) co-regulate stress-response and virulence genes, (ii) regulate genes encoding other regulators and (iii) regulate small RNAs.

Co-regulation of stress-response and virulence genes by alternative σ factors

Transcriptional analyses have provided clear evidence that several virulence and stress-response genes are co-regulated by either multiple alternative σ factors, or alternative σ factors and other transcriptional regulators. One important theme that has emerged from various transcriptional studies is that, in addition to σ^B-dependent transcription of *prfA* itself, several other virulence genes are also co-regulated by PrfA and σ^B (e.g. *inlA, inlB*, *bsh* and the *bilE* operon) (Figure 1). Interestingly, some of these genes (e.g. *inlA*, *bsh* and *bilEAB*) encode proteins that seem to have specific roles in the gastrointestinal stage of the interactions between *L. monocytogenes* and its hosts, indicating the need for interactions between PrfA and σ^B during the transition of *L. monocytogenes* from the non-host environment to the systemic stages of infection.

Genes regulated by more than one alternative σ factor (i.e. σ^B and σ^L) include those encoding cold-shock protein *cspD* and general-stress protein *lmo1601. cspD* is negatively regulated by σ^B under salt stress and by σ^L in late logarithmic-phase whereas *lmo1601*, which is positively regulated by σ^B under both salt stress and entry into stationary phase, is negatively regulated by σ^L [25,60]. σ^B and σ^L seem to modulate the expression of genes that are differentially expressed in intracellular bacteria, such as *ldh* and *pdhB*, which encode L-lactate dehydrogenase and a subunit of pyruvate dehydrogenase, respectively [25,47,60]. In addition to indicating interplay between σ^B and σ^L, these results also indicate that σ^B and σ^L control and fine-tune the expression of genes that is important for coping with physiological stresses and metabolic requirements in *L. monocytogenes*, including during host infection.

σ^B also co-regulates stress-response and virulence genes with negative regulators such as CtsR and HrcA, which both contribute to the regulation of expression of heat-shock genes

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and genes important for virulence [66,67] (Figure 1). Specific examples include: (i) the σ^B and CtsR co-regulation of genes encoding Clp proteins, which have endopeptidase and chaperone functions; and (ii) the σ^B and HrcA co-regulation of heat-shock proteins DnaKJ and GroESL, which act as chaperones. At least one gene (i.e. *clpP*) is co-regulated by σ^B, CtsR and σ^L. Furthermore, in addition to co-regulation by σ^B and σ^L, the expression of *cspD* is also positively regulated by HrcA. Networks between different transcriptional regulators, including alternative σ factors, thus seem to contribute to fine-tuning gene expression under various different stress conditions.

Regulation by alternative σ factors of genes encoding other regulators

Regulatory interactions among alternative σ factors and other regulators in *L. monocytogenes* are not limited to co-regulation of genes encoding effector proteins. Alternative σ factors also regulate the transcription of genes that encode proteins with regulatory functions, including other transcriptional regulators. One particularly intriguing and important interaction is the regulation of *prfA* transcription by σ^B (Box 2).

Emerging evidence indicates that σ^B contributes to the control of negative regulators CtsR and HrcA [66,67] and, hence, to the regulation of heat-shock proteins. σ^B seems to directly regulate HrcA through a σ^B promoter upstream of *hrcA*. Expression of *hrcA* from the σ^B-dependent promoter occurs in logarithmic-phase *L. monocytogenes* exposed to environmental stress (0.3 M NaCl), which indicates that the role of σ^B in *hrcA* expression might be growth-phase dependent [67]. σ^B seems to regulate CtsR indirectly. From a promoter upstream of *mcsA*, σ^B regulates transcription of the *mcsA-mcsB-clpC* operon, which encodes a set of modulators of CtsR activity [66]. In *B. subtilis*, McsA helps stabilize CtsR. Post-translational modification of CtsR by McsB results in release of CtsR from the operators of repressed genes. Consequently, released CtsR is targeted for degradation by the Clp proteins [68,69].

Contributions of alternatives σ factors to regulation of small RNAs

σ factors regulate small RNAs (sRNAs) directly by controlling their transcription. However, alternative σ factors also can regulate sRNAs indirectly by controlling the expression of binding proteins and chaperones such as Hfq. In *E. coli* and other bacteria, Hfq has two major roles: (i) pairing sRNA to targeted mRNA, resulting in mRNA inhibition, and (ii) binding directly to mRNA to stabilize the transcript. Hfq has been identified in many bacterial pathogens, including *Vibrio cholerae* and *Salmonella* Typhimurium, and contributes to virulence. In *L. monocytogenes*, Hfq contributes to stress response in the presence of salt and ethanol, in addition to contributing to virulence in mice, although no virulence role is apparent in tissue culture models [70]. In *E. coli*, Hfq regulates the translation of general-stress σ factor RpoS mRNA (reviewed in Ref. [71]). By contrast, *hfq* is regulated by the general-stress factor σ^B in *L. monocytogenes* [70] and by RpoS in *Legionella pneumophilia*, the causative agent of legionellosis [72]. Taken together, these findings indicate that, in addition to regulating gene expression at the level of transcription, alternative σ factors seem to exert post-transcriptional control indirectly through sRNAs and Hfq.

Concluding remarks

Developing an improved understanding of the roles of alternative σ factors within complex regulatory networks under the myriad environmental conditions that *L. monocytogenes* might encounter in its natural life represents a considerable challenge. Clearly, we are only starting to understand the roles that alternative σ factors have in enabling *L. monocytogenes* to thrive as a ‘Jekyll and Hyde’ in both host and non-host environments. Initial microarray

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studies indicate that the roles of stress-response regulators, and of σ^B in particular, differ across *L. monocytogenes* strains [73], indicating that the diversity of stress-response systems, even within a given species, is considerable. This is supported further by the observation that one alternative σ factor (σ^C) seems to be present only in a phylogenetically distinct lineage of *L. monocytogenes.* Taken together, the work in this field predicts that the roles of alternative σ factors in bacterial stress response and virulence will differ among strains within the same species. Therefore, considerable work remains to explore crucial regulatory networks present across the phylogenetic diversity of *L. monocytogenes*.

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References

1. Gray MJ, et al. How the bacterial pathogen *Listeria monocytogenes* mediates the switch from environmental Dr. Jekyll to pathogenic M_r Hyde. Infect Immun. 2006; 74:2505–2512. [PubMed: 16622185]
2. Lecuit M. Human listeriosis and animal models. Microbes Infect. 2007; 9:1216–1225. [PubMed: 17720601]
3. Farber JM, Losos JZ. *Listeria monocytogenes*: a foodborne pathogen. CMAJ. 1988; 138:413–418. [PubMed: 3124948]
4. Mead PS, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999; 5:607–625. [PubMed: 10511517]
5. Vazquez-Boland JA, et al. *Listeria* pathogenesis and molecular virulence determinants. Clin Micro Rev. 2001; 14:584–640. [PubMed: 11432815]
6. Jensen VB, et al. Interactions of the invasive pathogens *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Shigella flexneri* with M cells and murine Peyer's patches. Infect Immun. 1998; 66:3758–3766. [PubMed: 9673259]
7. Sauders, BD.; Wiedmann, M. Ecology of *Listeria* species and *L. monocytogenes* in the natural environment. In: Ryser, ET.; Marth, EH., editors. Listeria, Listeriosis, and Food Safety. 3. CRC Press; 2007. p. 21-53.
8. Wiedmann M, et al. Ribotype diversity of *Listeria monocytogenes* strains associated with outbreaks of listeriosis in ruminants. J Clin Microbiol. 1996; 34:1086–1090. [PubMed: 8727881]
9. Gombas DE, et al. Survey of *Listeria monocytogenes* in ready-to-eat foods. J Food Prot. 2003; 66:559–569. [PubMed: 12696677]
10. Rocourt, J.; Buchrieser, C. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification. In: Ryser, ET.; Marth, EH., editors. Listeria, Listeriosis, and Food Safety. 3. CRC Press; 2007. p. 1-20.
11. Wiedmann M, et al. General stress transcription factor σ^B and its role in acid resistance and virulence of *Listeria monocytogenes*. J Bacteriol. 1998; 180:3650–3656. [PubMed: 9658010]
12. Begley M, et al. Bile stress response in *Listeria monocytogenes* LO28: adaptation, cross-protection, and identification of genetic loci involved in bile resistance. Appl Environ Microbiol. 2002; 8:6005–6012. [PubMed: 12450822]
13. Karatzas KA, Bennik MH. Characterization of a *Listeria monocytogenes* Scott A isolate with high tolerance towards high hydrostatic pressure. Appl Environ Microbiol. 2002; 68:3183–3189. [PubMed: 12088993]
14. Conte MP, et al. Effect of acid adaptation on the fate of *Listeria monocytogenes* in THP-1 human macrophages activated by γ interferon. Infect Immun. 2002; 70:4369–4378. [PubMed: 12117947]

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15. Ferreira A, et al. Role of *Listeria monocytogenes* σ^B in survival of lethal acidic conditions and in the acquired acid tolerance response. Appl Environ Microbiol. 2003; 69:2692–2698. [PubMed: 12732538]
16. Kazmierczak MJ, et al. Alternative σ factors and their roles in bacterial virulence. Micro Mol Biol Rev. 2005; 69:527–543.
17. Magnusson LU, et al. ppGpp: a global regulator in *Escherichia coli*. Trends Microbiol. 2005; 13:236–242. [PubMed: 15866041]
18. Costanzo A, et al. ppGpp and DksA likely regulate the activity of the extracytoplasmic stress factor σ^E in *Escherichia coli* by both direct and indirect mechanisms. Mol Microbiol. 2008; 67:619–632. [PubMed: 18086212]
19. Stragier P, Losick R. Molecular genetics of sporulation in *Bacillus subtilis*. Annu Rev Gen. 1996; 30:297–341.
20. Glaser P, et al. Comparative genomics of *Listeria* species. Science. 2001; 294:849–852. [PubMed: 11679669]
21. Xu J, et al. A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. Science. 2003; 299:2074–2076. [PubMed: 12663928]
22. Kunst F, et al. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature. 1997; 390:249–256. [PubMed: 9384377]
23. Abram F, et al. Proteomic analyses of a *Listeria monocytogenes* mutant lacking σ^B identify new components of the σ^B regulon and highlight a role for σ^B in the utilization of glycerol. Appl Environ Microbiol. 2008; 74:594–604. [PubMed: 18065622]
24. Kazmierczak MJ, et al. *Listeria monocytogenes* σ^B regulates stress response and virulence functions. J Bacteriol. 2003; 185:5722–5734. [PubMed: 13129943]
25. Raengpradub S, et al. Comparative analysis of the σ^B-dependent stress responses in *Listeria monocytogenes* and *Listeria innocua* strains exposed to selected stress conditions. Appl Environ Microbiol. 2008; 74:158–171. [PubMed: 18024685]
26. Hecker M, et al. SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. Annu Rev Microbiol. 2007; 61:215–236. [PubMed: 18035607]
27. Petersohn A, et al. Global analysis of the general stress response of *Bacillus subtilis*. J Bacteriol. 2001; 183:5617–5631. [PubMed: 11544224]
28. Bischoff M, et al. Microarray-based analysis of the *Staphylococcus aureus* σ^B regulon. J Bacteriol. 2004; 186:4085–4099. [PubMed: 15205410]
29. Wemekamp-Kamphuis HH, et al. 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. 2004; 70:3457–3466. [PubMed: 15184144]
30. Chaturongakul S, Boor KJ. RsbT and RsbV contribute to σ^B-dependent survival under environmental, energy and intracellular stress conditions in *Listeria monocytogenes*. Appl Environ Microbiol. 2004; 70:5349–5356. [PubMed: 15345420]
31. Kazmierczak MJ, et al. Contributions of *Listeria monocytogenes* σ^B and PrfA to expression of virulence and stress response genes during extra- and intracellular growth. Microbiology. 2006; 152:1827–1838. [PubMed: 16735745]
32. Cotter PD, et al. Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated with an ability to grow at low pH. Appl Environ Microbiol. 2005; 71:2832–2839. [PubMed: 15932974]
33. Becker LA, et al. Identification of the gene encoding the alternative σ factor σ^B from *Listeria monocytogenes* and its role in osmotolerance. J Bacteriol. 1998; 180:4547–4554. [PubMed: 9721294]
34. Sue D, et al. σ^B-dependent expression patterns of compatible solute transporter genes *opuCA* and *lmo1421* and a conjugated bile salt hydrolase *bsh* in *Listeria monocytogenes*. Microbiology. 2003; 149:3247–3256. [PubMed: 14600237]
35. Fraser KR, et al. The role of σ^B in regulating compatible solute uptake systems of *Listeria monocytogenes*: osmotic induction of *opuC* is σ^B–dependent. Appl Environ Microbiol. 2003; 69:2015–2022. [PubMed: 12676677]

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36. Cetin MS, et al. Regulation of transcription of compatible solute transporters by the general stress sigma factor, σ^B, in *Listeria monocytogenes*. J Bacteriol. 2004; 186:794–802. [PubMed: 14729706]
37. Moorhead SM, Dykes GA. The role of the *sigB* gene in the general stress response of *Listeria monocytogenes* varies between a strain of serotype 1/2a and a strain of serotype 4c. Curr Microbiol. 2003; 46:461–466. [PubMed: 12732955]
38. Hain T, et al. Temporal transcriptomic analysis of the *Listeria monocytogenes* EGD-e σ^B regulon. BMC Microbiol. 2008; 8:20. [PubMed: 18226246]
39. Ferreira A, et al. Role of σ^B in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes*. Appl Environ Microbiol. 2001; 67:4454–4457. [PubMed: 11571142]
40. Chaturongakul S, Boor KJ. σ_B activation under environmental and energy stress conditions in *Listeria monocytogenes*. Appl Environ Microbiol. 2006; 72:5197–5203. [PubMed: 16885265]
41. Dussurget O, et al. *Listeria monocytogenes* bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. Mol Microbiol. 2002; 45:1095–1106. [PubMed: 12180927]
42. Sleator RD, et al. A PrfA-regulated bile exclusion system (BilE) is a novel virulence factor in *Listeria monocytogenes*. Mol Microbiol. 2005; 55:1183–1195. [PubMed: 15686563]
43. McGann P, et al. The alternative sigma factor σ^B and the virulence gene regulator PrfA both regulate transcription of *Listeria monocytogenes* internalins. Appl Environ Microbiol. 2007; 73:2919–2930. [PubMed: 17337550]
44. Nadon CA, et al. σ^B contributes to PrfA-mediated virulence in *Listeria monocytogenes*. Infect Immun. 2002; 70:3948–3952. [PubMed: 12065541]
45. Schwab U, et al. The *Listeria monocytogenes prfA*P2 promoter is regulated by σ^B in a growth phase dependent manner. FEMS Microbiol Lett. 2005; 245:329–336. [PubMed: 15837390]
46. Sleator RD, et al. Analysis of the role of OpuC, an osmolyte transport system, in salt tolerance and virulence potential of *Listeria monocytogenes*. Appl Environ Microbiol. 2001; 67:2692–2698. [PubMed: 11375182]
47. Chatterjee SS, et al. Intracellular gene expression profile of *Listeria monocytogenes*. Infect Immun. 2006; 74:1323–1338. [PubMed: 16428782]
48. Joseph B, et al. Identification of *Listeria monocytogenes* genes contributing to intracellular replication by expression profiling and mutant screening. J Bacteriol. 2006; 188:556–568. [PubMed: 16385046]
49. Kim H, et al. σ^B contributes to *Listeria monocytogenes* invasion by controlling expression of *inlA* and *inlB*. Microbiology. 2005; 151:3215–3222. [PubMed: 16207905]
50. Garner MR, et al. σ^B contributes to *Listeria monocytogenes* gastrointestinal infection but not to systemic spread in the guinea pig infection model. Infect Immun. 2006; 74:876–886. [PubMed: 16428730]
51. Fouet A, et al. Characterization of the operon encoding the alternative σ^B factor from *Bacillus anthracis* and its role in virulence. J Bacteriol. 2000; 182:5036–5045. [PubMed: 10960085]
52. Britton RA, et al. Genome-wide analysis of the stationary-phase sigma factor (Sigma-H) regulon of *Bacillus subtilis*. J Bacteriol. 2002; 184:4881–4890. [PubMed: 12169614]
53. Rea RB, et al. Disruption of putative regulatory loci in *Listeria monocytogenes* demonstrates a significant role for Fur and PerR in virulence. Infect Immun. 2004; 72:717–727. [PubMed: 14742513]
54. Morikawa K, et al. A new staphylococcal σ factor in the conserved gene cassette: functional significance and implication for the evolutionary processes. Genes Cells. 2003; 8:699–712. [PubMed: 12875655]
55. Hadjifrangiskou M, et al. The alternative sigma factor σ^H is required for toxin gene expression by *Bacillus anthracis*. J Bacteriol. 2007; 189:1874–1883. [PubMed: 17189374]
56. Robichon D, et al. The *rpoN* (σ-54) gene from *Listeria monocytogenes* is involved in resistance to mesentericin Y105, an antibacterial peptide from *Leuconostoc mesenteroides*. J Bacteriol. 1997; 179:7591–7594. [PubMed: 9393729]

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57. Liu S, et al. Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Appl Environ Microbiol. 2002; 68:1697–1705. [PubMed: 11916687]

58. Chan YC, et al. Microarray-based characterization of the *Listeria monocytogenes* cold regulon in log- and stationary-phase cells. Appl Environ Microbiol. 2007; 73:6484–6498. [PubMed: 17720827]

59. Dalet K, et al. A σ^{54}-dependent PTS permease of the mannose family is responsible for sensitivity of *Listeria monocytogenes* to mesentericin Y105. Microbiology. 2001; 147:3263–3269. [PubMed: 11739758]

60. Arous S, et al. Global analysis of gene expression in an *rpoN* mutant of *Listeria monocytogenes*. Microbiology. 2004; 150:1581–1590. [PubMed: 15133119]

61. Okada Y, et al. The sigma factor RpoN (σ^{54}) is involved in osmotolerance in *Listeria monocytogenes*. FEMS Microbiol Lett. 2006; 263:54–60. [PubMed: 16958851]

62. Erickson JW, Gross CA. Identification of the σ^{E} subunit of *Escherichia coli* RNA polymerase: a second alternative σ factor involved in high-temperature gene expression. Genes Dev. 1989; 3:1462–1471. [PubMed: 2691330]

63. Wang QP, Kaguni JM. A novel sigma factor is involved in expression of the *rpoH* gene of *Escherichia coli*. J Bacteriol. 1989; 171:4248–4253. [PubMed: 2546916]

64. Zhang C, et al. Functional consequences of genome evolution in *Listeria monocytogenes*: the *lmo0423* and *lmo0422* genes encode σ^{C} and LstR, a lineage II-specific heat shock system. J Bacteriol. 2005; 187:7243–7253. [PubMed: 16237008]

65. Bennett HJ, et al. Characterization of *relA* and *codY* mutants of *Listeria monocytogenes*: identification of the CodY regulon and its role in virulence. Mol Microbiol. 2007; 63:1453–1467. [PubMed: 17302820]

66. Hu Y, et al. Phenotypic and transcriptomic analyses demonstrate interactions between the transcriptional regulator CtsR and σ^{B} in *Listeria monocytogenes*. Appl Environ Microbiol. 2007; 73:7967–7980. [PubMed: 17933929]

67. Hu Y, et al. Transcriptomic and phenotypic analyses suggest a network between the transcriptional regulators HrcA and σ^{B} in *Listeria monocytogenes*. Appl Environ Microbiol. 2007; 73:7981–7991. [PubMed: 17965207]

68. Kirstein J, et al. A tyrosine kinase and its activator control the activity of the CtsR heat shock repressor in *B. subtilis*. EMBO J. 2005; 24:3435–3445. [PubMed: 16163393]

69. Kruger E, et al. Clp-mediated proteolysis in gram-positive bacteria is autoregulated by the stability of repressor. EMBO J. 2001; 20:852–863. [PubMed: 11179229]

70. Christiansen JK, et al. The RNA-binding protein Hfq of *Listeria monocytogenes*: role in stress tolerance and virulence. J Bacteriol. 2004; 186:3355–3362. [PubMed: 15150220]

71. Gottesman S. The small RNA regulators of *Escherichia coli*: roles and mechanisms. Annu Rev Microbiol. 2004; 58:303–328. [PubMed: 15487940]

72. McNealy TL, et al. The Hfq homolog in *Legionella pneumophila* demonstrates regulation by LetA and RpoS and interacts with the global regulator CsrA. J Bacteriol. 2005; 187:1527–1532. [PubMed: 15687220]

73. Severino P, et al. Comparative transcriptome analysis of *Listeria monocytogenes* strains of the two major lineages reveals differences in virulence, cell wall, and stress response. Appl Environ Microbiol. 2007; 73:6078–6088. [PubMed: 17704270]

74. Becker LA, et al. Role of σ^{B} in adaptation of *Listeria monocytogenes* to growth at low temperature. J Bacteriol. 2000; 182:7083–7087. [PubMed: 11092874]

75. Moorhead SM, Dykes GA. Influence of *sigB* gene on the cold stress survival and subsequent recovery of two *Listeria monocytogenes* serotypes. Int J Food Microbiol. 2004; 91:63–72. [PubMed: 14967561]

76. Chan YC, et al. σ^{B}-dependent and σ^{B}-independent mechanisms contribute to transcription of *Listeria monocytogenes* cold stress genes during cold shock and cold growth. Appl Environ Microbiol. 2007; 73:6019–6029. [PubMed: 17675428]

77. Sue D, et al. σ^{B}-dependent gene expression and induction in *Listeria monocytogenes* during osmotic and acid stress conditions simulating the intestinal environment. Microbiology. 2004; 150:3843–3855. [PubMed: 15528669]

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78. Johansson J, et al. An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. Cell. 2002; 110:551–561. [PubMed: 12230973]
79. Freitag NE, Portnoy DA. Dual promoters of the *Listeria monocytogenes prfA* transcriptional activator appear essential *in vitro* but are redundant *in vivo*. Mol Microbiol. 1994; 12:845–853. [PubMed: 8052135]
80. Bischoff M, et al. Influence of a functional *sigB* operon on the global regulators *sar* and *agr* in *Staphylococcus aureus*. J Bacteriol. 2001; 183:5171–5179. [PubMed: 11489871]
81. Arvidson S, Tegmark K. Regulation of virulence determinants in *Staphylococcus aureus*. Int J Med Microbiol. 2001; 291:159–170. [PubMed: 11437338]
82. Milohanic E, et al. Transcriptome analysis of *Listeria monocytogenes* identifies three groups of genes differently regulated by PrfA. Mol Microbiol. 2003; 47:1613–1625. [PubMed: 12622816]
83. Mogk A, et al. The GroE chaperonin machine is a major modulator of the CIRCE heat shock regulon of *Bacillus subtilis*. EMBO J. 1997; 16:4579–4590. [PubMed: 9303302]

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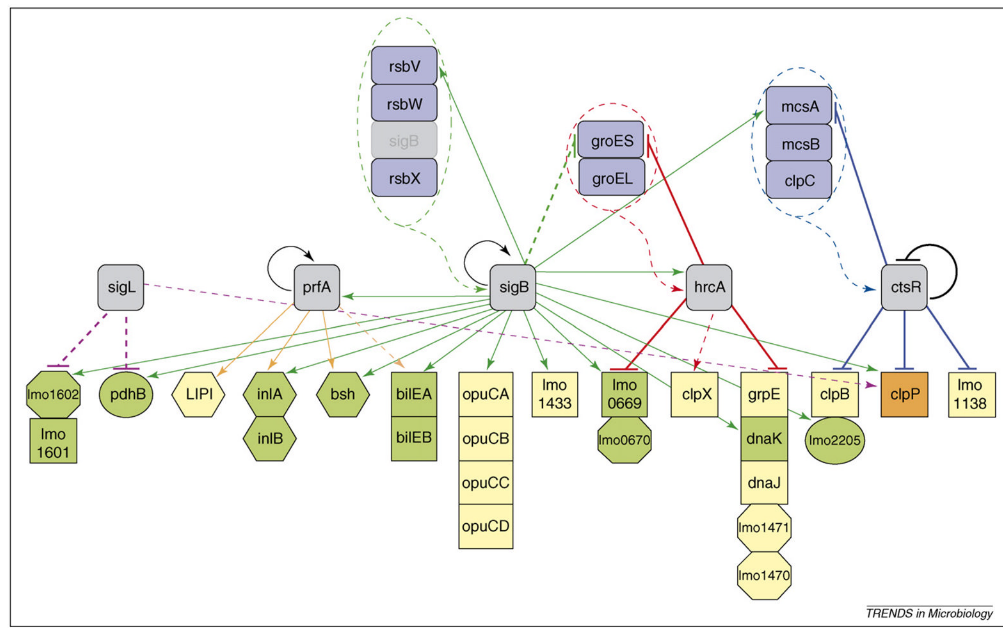

Figure 1.

Partial σ^L, PrfA, σ^B, HrcA and CtsR interaction network in *L. monocytogenes*. The network represented here is based on macroarray data for σ^L [60] and PrfA [82], and microarray data for σ^B [25], CtsR [66] and HrcA [67]; data for the *bilE* operon are based on Ref. [42]. The genes shown were selected specifically to highlight interactions between σ^L, PrfA, σ^B, HrcA and CtsR as identified by whole-genome transcriptome analyses and are only a subset of those regulated or co-regulated by the different regulator proteins. Different analytical approaches (e.g. through proteomic or metabolomic strategies) are likely to identify further additional interactions among these and other *L. monocytogenes* regulatory proteins. Color-coded arrows indicate genes regulated by σ^L (purple), PrfA (orange), σ^B (green), HrcA (red) or CtsR (blue). Solid lines indicate direct regulation of a gene by a given regulator as determined by the presence of a PrfA box, σ^B promoter, HrcA operator site or CtsR operator site; broken lines indicate indirect regulation. Target arrows (↓) indicate positive regulation by a given regulator; target stops (⊥) indicate negative regulation by a given regulator. Loops indicate autoregulation. Color-coded shapes identify (i) transcriptional regulators (gray rounded squares); (ii) genes that regulate a transcriptional regulator (blue rounded rectangles); and (iii) genes regulated by a single transcriptional regulator (yellow), two transcriptional regulators (green) or three transcriptional regulators (orange). Hexagons represent virulence genes (e.g. virulence genes directly regulated by PrfA [i.e. *plcA, hly, mpl, actA* and *plcB*] that comprise the *Listeria* pathogenicity island [LIPI]), squares represent stress-response genes (e.g. *grpE* encodes heat-shock protein GrpE), circles represent metabolism genes (e.g. *lmo2205* and *pdhB* encode a phosphoglyceromutase and a subunit of pyruvate dehydrogenase, respectively) and octagons represent genes encoding proteins of unknown or other function (e.g. *lmo1471* and *lmo1470* encode an unknown protein and a ribosomal protein methyltransferase, respectively). Genes arranged in vertical columns represent operons (e.g. *lmo0670*, which encodes a hypothetical protein, is in an operon with *lmo0669*, which encodes an oxidoreductase). The green arrow targeting σ^B indicates post-translational regulation of σ^B by RsbV, RsbW and RsbX, which are encoded by *rsb* (regulator of sigma *B*) genes *rsbV, rsbW* and *rsbX*, respectively [30]. The red arrow targeting HrcA indicates post-transcriptional regulation of HrcA by GroES and GroEL, based on evidence reported for *B. subtilis* [83]. The blue arrow targeting CtsR indicates

post-translational regulation of CtsR by McsA, McsB and ClpC, based on evidence reported for *B. subtilis* [68,69].

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Table 1

Listeria monocytogenes

σ^B contributions to stress resistance

Stress condition	σ^B-dependent genes	Phenotypic data supporting σ^B contributions	Refs
Acid	*gadA* and *gadCB* operon	Survival of Δ*sigB* strain is five logs lower than wild type when logarithmic- phase cells are exposed to pH 2.5 for 1 h	[29]
Osmotic	*betL*, *gbuA, bilEAB* and *opuCABCD*	Reduced ability to use carnitine and betaine as osmoprotectants in Δ*sigB* strain	[35]
Oxidative	*lmo0669*[a] and *lmo1433*	Survival of stationary-phase Δ*sigB* strain is one log lower than wild type when exposed to 13 mM CHP for 15 min	[30]
Energy	*opuCA* and carbon metabolism genes (e.g. *ldh*)	Viability of Δ*sigB* strain is impaired when glucose is limiting, upon entry into stationary phase and under conditions that deplete intracellular ATP	[30]
Cold or freezing	*ltrC (low-temperature requirement C)*	σ^B is not required during growth in rich media at 4 °C or for survival after freeze–thaw cycles, but contributes to growth in minimal media and in meats at low temperatures	[29,74–76]
High hydrostatic pressure	Not determined	Survival of Δ*sigB* strain is four logs lower than wild type at 350 MPa after 28 min	[29]
Arsenate	*lmo2230* (encodes a protein similar to arsenate reductase)	Not determined	[24,25]

[a] *lmo0669* might contribute to the acid-stress response at pH 4.5 [77].