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Listeria monocytogenes — from saprophyte to intracellular pathogen

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Abstract

Listeria monocytogenes is a bacterium that lives in the soil as a saprophyte but is capable of making the transition into a pathogen following its ingestion by susceptible humans or animals. Recent studies suggest that *L. monocytogenes* mediates its saprophyte-to-cytosolic-parasite transition through the careful modulation of the activity of a virulence regulatory protein known as PrfA, using a range of environmental cues that include available carbon sources. In this Progress article we describe the regulation of PrfA and its role in the *L. monocytogenes* transition from the saprophytic stage to the virulent intracellular stage.

Humans are surrounded by an incredible abundance of diverse microorganisms. Estimates indicate that the flora living in the human mouth (>500 species) and gastrointestinal tract (>2,000 species) as well as on the skin (>180 species)[1–3] are vastly diverse, and that soil contains 10^6–10^7 prokaryotic species per gram[4]. Given their vast abundance and diversity, it is reassuring to note that only a tiny proportion of these microorganisms are known to cause human disease. Recognizing that the environment provides a substantial reservoir of microorganisms, we pose two questions: what separates the potential pathogens from the non-pathogens and what types of adaptations enable a soil saprophyte to become a human pathogen?

The bacterial pathogen *Listeria monocytogenes* is well adapted to both life in the soil and life in the cytosol of eukaryotic host cells. This Gram-positive saprophyte is ubiquitous in the environment, where it is thought to live off decaying plant material[5]. Following ingestion by a susceptible human, the bacterium is capable of making the transition to a physiological state that promotes bacterial survival and replication in host cells[6]. In healthy individuals, the disease caused by *L. monocytogenes* is usually restricted to a self-limiting gastroenteritis; however, in immunocompromised individuals and pregnant women, the bacterium is capable of causing systemic infections that lead to meningitis, encephalitis and, in the case of pregnant women, infection of the developing fetus, which can lead to abortion, stillbirth or neonatal infections[7]. The lifestyle switch to intracellular pathogen includes increases in the expression of gene products that are known to promote cell-to-cell spread and bacterial replication in the host cytosol; these gene products are generally expressed at low levels outside of the host[8]. How does *L. monocytogenes* implement the transition from life in the soil to life in the cell?

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Bacteria must be capable of distinguishing the myriad of environmental cues encountered both inside and outside host cells and of correctly interpreting the signals so as to express gene products that promote survival in the appropriate location. Below, we discuss recent progress that has been made towards understanding how *L. monocytogenes* mediates the switch between its disparate lifestyles.

Life in the outside environment

L. monocytogenes has been isolated from soil, silage, groundwater, sewage and vegetation[9] (FIG. 1). Whether it is associated with a lower eukaryotic host, such as a fungus, protist or nematode, has not been clearly established, although it is anticipated that the microorganism must frequently encounter these potential predators[10]. Substantial attention has been given to the ability of *L. monocytogenes* to survive in food processing plants, where it can withstand environmental stresses that normally serve to limit bacterial growth, such as metal ions, high salt, fluctuations in pH and low temperature[9]. A large number of gene products that are associated with various forms of stress resistance mechanisms (including resistance to acid, osmotic and temperature stress) have been identified in *L. monocytogenes*; many of these are regulated by the alternative sigma factor σ^B, which directs RNA polymerase to target stress-responsive gene promoters[11]. Although *L. monocytogenes* does not form spores, it can become firmly established in food processing environments and can persist for long periods of time and even years[12]. *L. monocytogenes* is therefore clearly built to last in many different habitats.

Life in the mammalian host

As a pathogen, *L. monocytogenes* infects a wide range of host species and host cell types[13,14]. The primary route of infection is across the intestinal epithelium after consumption of contaminated food products by the host. Both intragastric and intravenous models of *L. monocytogenes* infection exist for mice, guinea pigs, gerbils and monkeys[13,15–18]. Following entry into the bloodstream, most of the bacteria end up in the liver and spleen, owing to trafficking by macrophages. Unless their replication is controlled by an effective host innate immune response, the bacteria escape from immune clearance and continue to divide and replicate[19]. Host survival then depends on the development of an effective adaptive immune response; otherwise, the bacteria re-enter the bloodstream to cause potentially fatal systemic or central nervous system infections. The ability of *L. monocytogenes* to replicate in the cytosol of infected host cells and to spread from cell to cell enables it to avoid humoral immune responses[20].

A number of bacterial surface proteins, including the internalins InlA and InlB, have been shown to contribute to bacterial invasion of host cells[14] (FIG. 1). InlA binds E-cadherin, a host cell adhesion molecule, whereas InlB binds to the hepatocyte growth factor (HGF) receptor, Met; binding to these receptors enables *L. monocytogenes* to gain entry into host cells through the exploitation of the host endocytic machinery[21]. Once internalized, *L. monocytogenes* mediates its escape from the membrane-bound vacuole by secreting a pore-forming cytolysin, known as listeriolysin O (LLO), and two phospholipases, which work together to break down the phagosome in which it resides[8,22–26]. Within the host cell cytosol, the bacteria replicate using nutrients that are acquired from the host (including hexose phosphate sugars that are acquired through the bacterial hexose phosphate transporter, Hpt, as well as lipoic acid and peptides)[27–29]. *L. monocytogenes* then moves through the cell and into adjacent cells using actin polymerization as a motility force, which it directs through its surface protein actin assembly-inducing protein (ActA)[21]. The bacteria enter adjacent cells and secrete LLO and the broad-specificity phosphatidylcholine phospholipase C (PC-PLC) to escape from the double-membraned secondary vacuoles that are formed as a result of cell-to-cell spread[22,24,30].

Regulating the outside-to-inside switch

Nearly all of the gene products that contribute to bacterial invasion, cytosolic entry and growth, intracellular motility and spread to adjacent cells are regulated by the transcriptional regulator PrfA[6,31] (FIG. 1; TABLE 1). The core PrfA regulon encompasses 10 genes that are directly regulated by PrfA, and up to 145 additional putative PrfA-regulated genes have been implicated by microarray expression data or proteomic analyses[8,32]. PrfA can also regulate genes that contribute to bile resistance, an attribute that may facilitate *L. monocytogenes* persistence in the gall bladder[33–35]. PrfA induces the expression of a bile salt hydrolase (encoded by *bsh*) as well as a bile exclusion system, both of which contribute to bacterial survival in the intestine[36]. *L. monocytogenes* mutants that lack a functional PrfA do not replicate in infected cells and are 100,000-fold less virulent than wild-type strains in mouse models of infection[37].

There are multiple mechanisms for regulating both the expression and the activity of PrfA. In addition to transcriptional regulation[38], the expression of PrfA is regulated by an RNA thermosensor mechanism that facilitates protein translation at 37 °C[39]; however, an increase in environmental temperature alone is not sufficient to induce PrfA-dependent gene expression in bacteria grown in broth culture. PrfA activity is evident at 25 °C in bacteria growing within insect cells, suggesting that a host cell-derived signal can activate virulence gene expression40–43. In broth cultures of bacteria grown at 37 °C, PrfA is present but inactive44, and most PrfA-regulated gene products are highly induced on bacterial entry into the host cytosol[8]. PrfA is a member of the cyclic AMP receptor protein (crp) family of transcriptional activators[45,46], many members of which require the binding of small molecular cofactors for full activity47. It has therefore been postulated that PrfA activation occurs through the binding of a small molecular cofactor following host cell entry. The identity of this putative PrfA cofactor is not yet known, but intriguing links exist between carbon metabolism and PrfA-dependent gene expression, which suggests that nutrient availability in the host may serve as a signal to *L. monocytogenes* as to its intracellular location.

It has been known for more than a decade that bacterial growth in the presence of readily metabolized carbohydrates, such as glucose and cellobiose, dramatically inhibits the expression of PrfA-dependent gene products[48,49]. It was subsequently observed that *L. monocytogenes* can use glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate and mannose-6-phosphate as carbon sources and that metabolism of these sugars does not lead to the repression of virulence gene expression[50]. These observations suggest that when in the cytosol of host cells, *L. monocytogenes* encounters and consumes hexose phosphates as carbon sources while maintaining a high level of PrfA-dependent virulence gene expression. By contrast, carbon sources that are commonly found in the outside environment, such as the plant sugar cellobiose, may function as signals to *L. monocytogenes* that it is in an environment where PrfA-dependent gene expression is not required[48,51].

The available nutrients could therefore serve as the signal to *L. monocytogenes* as to whether it should live as a saprophyte or as a pathogen. When bacteria that were initially grown in rich medium enter host cells, they switch from using glycolysis to using the pentose phosphate cycle as the predominant pathway for sugar metabolism[52]. phosphorylated glucose that is derived from host cell glycogen and a three-carbon compound have both been implicated as intracellular carbon sources for *L. monocytogenes*52,[53]. By contrast, when the bacterium is outside a host cell, PrfA-dependent gene expression is repressed, owing to the uptake of carbohydrates by the phosphoenolpyruvate phosphotransferase system (PTS)[54]. A model describing the potential relationship between carbon metabolism and PrfA activity has been recently suggested by Joseph *et al.*[55] and Stoll *et al.*[56] (FIG. 2). These authors observed that the repression of PrfA-dependent gene expression correlates directly with the phosphorylation

status of PTS permeases (enzyme II, or EII components, of the PTS complex). In the presence of PTS-dependent sugars, the transport of these carbohydrates across the bacterial membrane results in the transfer of a phosphate group from the PTS EII domain A (EIIA) to the incoming sugar and the subsequent accumulation of the non-phosphorylated form of EIIA, which in turn correlates with a decrease in PrfA-dependent gene expression[52] (FIG. 2a). By contrast, the phosphorylated form of EIIA accumulates during bacterial growth on non-PTS-dependent carbon sources and this form of the enzyme is associated with high levels of expression of PrfA-dependent gene products[52] (FIG. 2b). On the basis of these observations, the authors proposed that the sugar-specific EIIA component of PTS, in its non-phosphorylated state, serves to bind and sequester PrfA, thereby keeping the regulator functionally inactive and preventing the induction of virulence gene expression (FIG. 2a). In the presence of non-PTS-dependent carbon sources, such as hexose phosphates or glycerol, the lack of PTS-dependent sugar transport results in the accumulation of the phosphorylated form of EIIA and the release of PrfA, which is then fully active and able to induce target gene expression[52,56] (FIG. 2b).

Although several features of this model are attractive, it does suggest that PrfA may differ from other crp family members by not requiring the binding of a small signal molecule or cofactor for full activity[47]. The model proposed by Joseph *et al.* suggests that PrfA would be fully active following its release from EIIA, without either a signal molecule or post-translational modification[55]. However, both structural and functional analyses of wild-type and mutationally activated PrfA proteins (PrfA* mutants; discussed below) suggest that the increase in PrfA activity is caused by conformational changes that promote higher-affinity DNA binding. It has been argued that these conformational changes occur as a result of cofactor binding in a structurally defined binding pocket[57–60]. It is possible that the phosphorylated PTS permeases of *L. monocytogenes* stimulate the synthesis of a cofactor or second messenger that serves to activate PrfA (FIG. 2c). In *Escherichia coli*, glucose-specific PTS EIIA (EIIAGlc)-phosphate stimulates adenylyl cyclase to produce the crp cofactor cAMP[54]. Alternatively, it is possible that activation of PrfA by a cytosol-induced signal is required as an additional step following the release of PrfA by EIIA (FIG. 2c). The combination of cofactor activation and the sequestration and release of PrfA by EIIA may serve to more fully restrict PrfA activity to the correct environmental location.

Impact of *prfA** mutations

Although the signal or cofactor that results in PrfA activation remains unknown, the identification of mutations in *prfA* that result in its constitutive activation (*prfA** mutations) has allowed the investigation of PrfA activity to progress. Since the first identification of a *prfA** mutation, by Ripio *et al.* in 1997 (REF. 60), several amino acid substitutions in PrfA have been described that result in the activation of PrfA even in broth culture[59–64] (FIG. 3). Structural analysis of the original PrfA* mutant, PrfA G145S, indicates that this mutation results in a repositioning of the PrfA helix–turn–helix DNA binding region in comparison to the wild-type structure[57]. Surface plasmon resonance experiments have shown that there is an 18-fold increase in the DNA binding affinity of this PrfA* mutant; it is thought that this increase is attributable to the helix–turn–helix shift[57]. Additional structural alterations were observed for PrfA G145S in comparison to wild-type PrfA, but the implications of these changes are not yet clear. Mutations conferring *prfA** phenotypes are not equivalent with respect to their effects on PrfA-dependent gene expression; some mutations, for example E77K and G155S, confer mid-level activity changes (a 10- to 40-fold increase compared with the wild type), whereas others, such as L140F, G145S and Y63c, result in substantially greater (>200-fold) levels of activation[32,59]. Although detailed structural analyses of these mutants have not yet been undertaken (with the exception of G145S), experiments designed to identify structural alterations based on limited protease digestion patterns of purified protein have indicated that all of the mutant proteins exhibit conformational changes in comparison with the wild

type59. A striking correlation was also observed between the DNA binding affinity of selected purified PrfA* proteins for target promoters, as measured by gel electromobility shift assays, and the levels of PrfA-dependent gene expression in broth-grown cultures. The *prfA** alleles that conferred the highest levels of PrfA-dependent gene expression also exhibited the largest increases in DNA binding affinity[59,65]. These data imply that a conformational change in PrfA structure is required for full protein activity.

The PrfA* regulon and protein secretion

There are currently ten genes that have been shown to be directly regulated by PrfA, and that are therefore considered to be `core' members of the PrfA regulon[8] (TABLE 1). PrfA induces the expression of a set of genes, the products of which are required for bacterial entry into host cells (*inlA* and *inlB*), escape from the phagosome (*hly*, *plcA* and *plcB*), growth in the cytosol (*hpt*) and cell-to-cell spread (*actA*, *plcB*, *mpl* and *hly*). Additional gene products (~145) have been identified that could be regulated by PrfA, based on transcriptome profiling; however, a direct demonstration of the regulation of these gene products by PrfA has not yet been reported[8]. Genes that are found to have increased expression as a result of PrfA activation include those with products that are predicted to function in carbohydrate transport, protein folding and protein secretion, as well as several predicted proteins of unknown function66. Most notable was the induction of a number of genes that are associated with *L. monocytogenes* stress responses, many of which lack obvious PrfA binding sites (and are therefore presumably indirectly regulated by PrfA) and are directly regulated by the stress-responsive σ^B. There seems to be an intimate link between stress resistance and virulence gene expression, which may reflect the interplay between the PrfA and σ^B regulons that promotes bacterial survival in the stressful environments that are found in the host[67–69]. A new study by Toledo-Arana *et al.*, which analyses the transcript profiles of the entire *L. monocytogenes* genome, has implicated σ^B regulation in bacterial survival in the intestine and PrfA regulation in promoting bacterial replication in the blood[70].

Recently, *prfA** strains were used as tools to explore the changes that occur in the secreted protein profiles of *L. monocytogenes* as a result of PrfA activation. Bacterial virulence factors are often either secreted or localized on the bacterial surface, where they promote interaction and engagement with host cell components. A comparison was carried out among the secreted proteins that were isolated from the supernatants of wild-type, Δ*prfA* and *prfA** mutant bacteria to identify potential virulence-associated proteins with production and/or secretion that was directly or indirectly dependent on PrfA activation[32]. Seventeen proteins were identified that are differentially secreted as a result of PrfA activation, including several known virulence factors (LLO, ActA and PC-PLC), three putative ABC transporter components, four putative cell wall-modifying enzymes, two antigenic lipoproteins and two chaperones that are involved in protein secretion[32]. Mutational inactivation of a subset of these proteins indicated that there may be a role for each tested protein in *L. monocytogenes* virulence[32]. Many of the genes that encode these proteins did not contain recognizable PrfA binding sites, suggesting that although the secretion of these proteins depends on the activation state of PrfA, the proteins themselves are not directly regulated by PrfA. PrfA activation therefore has a far-reaching impact on the expression and activity of multiple factors that contribute to *L. monocytogenes* pathogenesis.

PrfA as a switch between lifestyles

It is clear that the mutational activation of PrfA has pleiotrophic effects on *L. monocytogenes* physiology. Strains containing *prfA** alleles are hyperinvasive for tissue culture cells and seem to lyse the phagosomal membrane and to associate with host cell actin more quickly than wild-type *L. monocytogenes*[71]. *prfA** strains are fully virulent or even hypervirulent following intravenous inoculation of mice; as PrfA is constitutively activated in

these strains, this would suggest that there is no need to de-activate or down-modulate the activity of PrfA once the protein is activated following bloodstream entry[61,72] (J. Bruno and N.F., unpublished observations). The basis of the hypervirulent phenotypes for *prfA** mutants following entry into the bloodstream is not known, nor has it yet been shown whether *prfA** mutants remain hypervirulent following intragastric inoculation. It seems plausible that the enhanced expression of PrfA-dependent gene products that are required for bacterial invasion, phagosome escape and actin-based motility results in a kind of virulence priming that contributes to the hypervirulent phenotype that is observed *in vivo*. Overexpression of some PrfA-dependent gene products, such as LLO, has been shown to reduce bacterial virulence *in vivo*, owing to the lysis of host cells and the elimination of the *L. monocytogenes* cytosolic replication niche[22]. *prfA** strains therefore seem to mediate the enhanced expression of virulence-associated gene products while avoiding the detrimental effects of their overexpression. Interestingly, virulence-attenuated *L. monocytogenes* strains containing *prfA** mutations have shown considerable promise as vaccine vectors (BOX 1).

Why, then, is PrfA activity so carefully regulated? The answer seems to reside in the requirement for *L. monocytogenes* to balance life in the host with life in the outside environment. PrfA* strains exhibit pronounced swimming motility defects[61], and recent evidence from broth culture competition assays indicates that the mutant strains are out-competed in broth culture by wild-type strains[73] (J. Bruno and N.F., unpublished observations). The crucial importance of PrfA activation for *L. monocytogenes* virulence is further demonstrated by the phenotype of a *prfA* mutant that is apparently locked into a low activation state. *prfA* Y154C mutants exhibit a modest (fourfold) increase in PrfA-dependent gene expression when grown in broth culture, but fail to activate the high-level gene expression that is required for actin-based motility and cell-to-cell spread following entry into the cytosol[58]. As a result, strains containing the *prfA* Y154C mutation are severely attenuated in mice (over 150-fold less virulent than wild-type strains)[58]. *L. monocytogenes* must therefore retain the ability to control PrfA activity in order to respond to environmental cues and optimize its fitness in highly diverse settings.

Outlook

PrfA clearly plays an important part in mediating the *L. monocytogenes* transition from saprophyte to pathogen. Functional analyses of *prfA* mutants indicate that *L. monocytogenes* must carefully regulate the activation status of PrfA to optimize its fitness and to retain its ability to replicate under diverse environmental conditions. Shifting the activity balance towards full PrfA activation and virulence gene expression results in increased fitness when in the host but compromises the ability of the bacterium to obtain and use nutrients when outside infected animals. A molecular determination of how and why PrfA activation compromises the fitness of *L. monocytogenes* outside host cells should clarify which aspects of the bacterium's physiology are important for extracellular versus intracellular life and may define which types of genetic and metabolic alterations promote the transformation of a soil dweller into a cell invader.

If the way to *L. monocytogenes'* pathogenic heart is through its stomach, according to the available nutrients, then the capacity of *L. monocytogenes* to flourish in diverse environments remains a complex phenomenon and one that involves multiple regulatory circuits. In addition to carbon metabolism, an intimate relationship has been observed between stress response pathways and PrfA-dependent gene expression, and it has been proposed that the physiological stresses that are encountered by *L. monocytogenes* during its passage through the stomach and intestine serve to prime it for host cell invasion and intracellular replication[11,67–70]. On the basis of the diversity of lifestyles and habitats in which *L. monocytogenes* resides, one might speculate that, together, an ability to use a wide range of nutrient sources and a robust capacity

to withstand divergent stresses might serve to assist the development of a microorganism into a pathogen.

Box 1 | Mutationally activated *prfA** strains as vectors for vaccine delivery

The capacity of *Listeria monocytogenes* to efficiently gain access to the cytosol of infected host cells and to stimulate a robust T cell response has led to the development of *L. monocytogenes*-based vaccine vectors for a range of infectious agents and cancers[74–78]. As a means of increasing the efficiency of antigen delivery by attenuated *L. monocytogenes* strains, Lauer *et al.*[79] and Yan *et al.*[80] recently made use of *prfA** mutations to increase the expression of selected antigens that were placed under the control of PrfA-dependent promoters. Interestingly, the *prfA** mutation that elicited the most effective T cell responses for recombinant strains was the *prfA* G155S mutation, a *prfA** allele that results in mid-level PrfA activation[32,79,80]. Although antigen expression and secretion were increased in *prfA* G155S mutants grown in broth culture, this increase did not seem to fully account for the improved stimulation of host immune responses[79]. It is possible that the *prfA* G155S mutation results in the increased expression of additional *L. monocytogenes* factors that serve as adjuvants for an enhanced host immune response. Precisely how the mutational activation of PrfA influences host immune responses is worthy of further investigation.

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References

1. Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. Proc. Natl Acad. Sci. USA 2007;104:2927–2932. [PubMed: 17293459]
2. Sakamoto M, Umeda M, Benno Y. Molecular analysis of human oral microbiota. J. Periodont. Res 2005;40:277–285. [PubMed: 15853975]
3. Zoetendal EG, Rajilic-Stojanovic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut 2008;57:1605–1615. [PubMed: 18941009]
4. Gans J, Wolinsky M, Dunbar J. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 2005;309:1387–1390. [PubMed: 16123304]
5. Gray ML, Killinger AH. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev 1966;30:309–382. [PubMed: 4956900]
6. Freitag NE. From hot dogs to host cells: how the bacterial pathogen *Listeria monocytogenes* regulates virulence gene expression. Future Microbiol 2006;1:89–101. [PubMed: 17661688]
7. Drevets DA, Bronze MS. *Listeria monocytogenes*: epidemiology, human disease, and mechanisms of brain invasion. FEMS Immunol. Med. Microbiol 2008;53:151–165. [PubMed: 18462388]
8. Scortti M, Monzo HJ, Lacharme-Lora L, Lewis DA, Vazquez-Boland JA. The PrfA virulence regulon. Microbes Infect 2007;9:1196–1207. [PubMed: 17764998]
9. Thevenot D, Dernburg A, Vernozy-Rozand C. An updated review of *Listeria monocytogenes* in the pork meat industry and its products. J. Appl. Microbiol 2006;101:7–17. [PubMed: 16834586]
10. Hilbi H, Weber SS, Ragaz C, Nyfeler Y, Urwyler S. Environmental predators as models for bacterial pathogenesis. Environ. Microbiol 2007;9:563–575. [PubMed: 17298357]
11. Chaturongakul S, Raengpradub S, Wiedmann M, Boor KJ. Modulation of stress and virulence in *Listeria monocytogenes*. Trends Microbiol 2008;16:388–396. [PubMed: 18619843]

12. Lunden JM, Autio TJ, Korkeala HJ. Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. J. Food Prot 2002;65:1129–1133. [PubMed: 12117246]
13. Lecuit M. Human listeriosis and animal models. Microbes Infect 2007;9:1216–1225. [PubMed: 17720601]
14. Seveau S, Pizarro-Cerda J, Cossart P. Molecular mechanisms exploited by *Listeria monocytogenes* during host cell invasion. Microbes Infect 2007;9:1167–1175. [PubMed: 17761447]
15. Wollert T, et al. Extending the host range of *Listeria monocytogenes* by rational protein design. Cell 2007;129:891–902. [PubMed: 17540170]
16. Bakardjiev AI, Stacy BA, Fisher SJ, Portnoy DA. Listeriosis in the pregnant guinea pig: a model of vertical transmission. Infect. Immun 2004;72:489–497. [PubMed: 14688130]
17. Smith MA, et al. Dose-response model for *Listeria monocytogenes*-induced stillbirths in nonhuman primates. Infect. Immun 2008;76:726–731. [PubMed: 18070908]
18. Blanot S, et al. A gerbil model for rhombencephalitis due to *Listeria monocytogenes*. Microb. Pathog 1997;23:39–48. [PubMed: 9250779]
19. Zenewicz LA, Shen H. Innate and adaptive immune responses to *Listeria monocytogenes*: a short overview. Microbes Infect 2007;9:1208–1215. [PubMed: 17719259]
20. Pamer EG. Immune responses to *Listeria monocytogenes*. Nature Rev. Immunol 2004;4:812–823. [PubMed: 15459672]
21. Pizarro-Cerda J, Cossart P. Subversion of cellular functions by *Listeria monocytogenes*. J. Pathol 2006;208:215–223. [PubMed: 16362984]
22. Schnupf P, Portnoy DA. Listeriolysin O: a phagosome-specific lysin. Microbes Infect 2007;9:1176–1187. [PubMed: 17720603]
23. Kathariou S, Metz P, Hof H, Goebel W. Tn*916*-induced mutations in the hemolysin determinant affecting virulence of *Listeria monocytogenes*. J. Bacteriol 1987;169:1291–1297. [PubMed: 3029033]
24. Vazquez-Boland J, et al. Nucleotide sequence of the lecithinase operon of *Listeria monocytogenes* and possible role of lecithinase in cell-to-cell spread. Infect. Immun 1992;60:219–230. [PubMed: 1309513]
25. Mengaud J, Braun-Breton C, Cossart P. Identification of phosphatidylinositol-specific phospholipase C activity in *Listeria monocytogenes*: a novel type of virulence factor? Mol. Microbiol 1991;5:367–372. [PubMed: 1645839]
26. Camilli A, Goldfine H, Portnoy DA. *Listeria monocytogenes* mutants lacking phosphatidylinositol-specific phospholipase C are avirulent. J. Exp. Med 1991;173:751–754. [PubMed: 1847723]
27. Joseph B, Goebel W. Life of *Listeria monocytogenes* in the host cells' cytosol. Microbes Infect 2007;9:1188–1195. [PubMed: 17719818]
28. Marquis H, Bouwer HG, Hinrichs DJ, Portnoy DA. Intracytoplasmic growth and virulence of *Listeria monocytogenes* auxotrophic mutants. Infect. Immun 1993;61:3756–3760. [PubMed: 8359896]
29. O'Riordan M, Moors MA, Portnoy DA. *Listeria* intracellular growth and virulence require host-derived lipoic acid. Science 2003;302:462–464. [PubMed: 14564012]
30. Yeung PS, Na Y, Kreuder AJ, Marquis H. Compartmentalization of the broad-range phospholipase C activity to the spreading vacuole is critical for *Listeria monocytogenes* virulence. Infect. Immun 2007;75:44–51. [PubMed: 17060464]
31. Scortti M, et al. Coexpression of virulence and fosfomycin susceptibility in *Listeria*: molecular basis of an antimicrobial *in vitro–in vivo* paradox. Nature Med 2006;12:515–517. [PubMed: 16633349]
32. Port GC, Freitag NE. Identification of novel *Listeria monocytogenes* secreted virulence factors following mutational activation of the central virulence regulator, PrfA. Infect. Immun 2007;75:5886–5897. [PubMed: 17938228]
33. Begley M, Sleator RD, Gahan CG, Hill C. Contribution of three bile-associated loci, *bsh*, *pva*, and *btlB*, to gastrointestinal persistence and bile tolerance of *Listeria monocytogenes*. Infect. Immun 2005;73:894–904. [PubMed: 15664931]
34. Hardy J, et al. Extracellular replication of *Listeria monocytogenes* in the murine gall bladder. Science 2004;303:851–853. [PubMed: 14764883]

35. 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]
36. Gahan CG, Hill C. Gastrointestinal phase of *Listeria monocytogenes* infection. J. Appl. Microbiol 2005;98:1345–1353. [PubMed: 15916648]
37. Freitag NE, Rong L, Portnoy DA. Regulation of the *prfA* transcriptional activator of *Listeria monocytogenes*: multiple promoter elements contribute to intracellular growth and cell-to-cell spread. Infect. Immun 1993;61:2537–2544. [PubMed: 8388865]
38. 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]
39. Johansson J, et al. An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. Cell 2002;110:551. [PubMed: 12230973]
40. Cheng LW, Portnoy DA. *Drosophila* S2 cells: an alternative infection model for *Listeria monocytogenes*. Cell. Microbiol 2003;5:875–885. [PubMed: 14641173]
41. Cheng LW, et al. Use of RNA interference in *Drosophila* S2 cells to identify host pathways controlling compartmentalization of an intracellular pathogen. Proc. Natl Acad. Sci. USA 2005;102:13646–13651. [PubMed: 16157870]
42. Mansfield BE, Dionne MS, Schneider DS, Freitag NE. Exploration of host–pathogen interactions using *Listeria monocytogenes* and *Drosophila melanogaster*. Cell. Microbiol 2003;5:901–911. [PubMed: 14641175]
43. Agaisse H, et al. Genome-wide RNAi screen for host factors required for intracellular bacterial infection. Science 2005;309:1248–1251. [PubMed: 16020693]
44. Renzoni A, Klarsfeld A, Dramsi S, Cossart P. Evidence that PrfA, the pleitropic activator of virulence genes in *Listeria monocytogenes*, can be present but inactive. Infect. Immun 1997;65:1515–1518. [PubMed: 9119495]
45. Lampidis R, Gross R, Sokolovic Z, Goebel W, Kreft J. 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 1994;13:141–151. [PubMed: 7984088]
46. Vega Y, et al. Functional similarities between the *Listeria monocytogenes* virulence regulator PrfA and cyclic AMP receptor protein: the PrfA* (Gly145Ser) mutation increases binding affinity for target DNA. J. Bacteriol 1998;180:6655–6660. [PubMed: 9852011]
47. Korner H, Sofia HJ, Zumft WG. Phylogeny of the bacterial superfamily of Crp–Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. FEMS Microbiol. Rev 2003;27:559–592. [PubMed: 14638413]
48. Milenbachs AA, Brown DP, Moors M, Youngman P. Carbon-source regulation of virulence gene expression in *Listeria monocytogenes*. Mol. Microbiol 1997;23:1075–1085. [PubMed: 9076743]
49. Park SF, Stewart GSAB, Kroll RG. The use of bacterial luciferase for monitoring the environmental regulation of expression of genes encoding virulence factors in *Listeria monocytogenes*. J. Gen. Microbiol 1992;138:2619–2627. [PubMed: 1487729]
50. Chico-Calero I, et al. Hpt, a bacterial homolog of the microsomal glucose-6-phosphate translocase, mediates rapid intracellular proliferation in *Listeria*. Proc. Natl Acad. Sci. USA 2002;99:431–436. [PubMed: 11756655]
51. Park SF, Kroll RG. Expression of listeriolysin and phosphatidylinositol-specific phospholipase C is repressed by the plant-derived molecule cellobiose in *Listeria monocytogenes*. Mol. Microbiol 1993;8:653–661. [PubMed: 8332058]
52. 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]
53. Eylert E, et al. Carbon metabolism of *Listeria monocytogenes* growing inside macrophages. Mol. Microbiol 2008;69:1008–1017. [PubMed: 18627458]
54. Gorke B, Stulke J. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nature Rev. Microbiol 2008;6:613–624. [PubMed: 18628769]
55. Joseph B, et al. Glycerol metabolism and PrfA activity in *Listeria monocytogenes*. J. Bacteriol 2008;190:5412–5430. [PubMed: 18502850]

56. Stoll R, Mertins S, Joseph B, Muller-Altrock S, Goebel W. Modulation of PrfA activity in *Listeria monocytogenes* upon growth in different culture media. Microbiology 2008;154:3856–3876. [PubMed: 19047753]
57. Eiting M, Hageluken G, Schubert WD, Heinz DW. The mutation G145S in PrfA, a key virulence regulator of *Listeria monocytogenes*, increases DNA-binding affinity by stabilizing the HTH motif. Mol. Microbiol 2005;56:433–446. [PubMed: 15813735]
58. Miner MD, Port GC, Bouwer HG, Chang JC, Freitag NE. A novel *prfA* mutation that promotes *Listeria monocytogenes* cytosol entry but reduces bacterial spread and cytotoxicity. Microb. Pathog 2008;45:273–281. [PubMed: 18675335]
59. Miner MD, Port GC, Freitag NE. Functional impact of mutational activation on the *Listeria monocytogenes* central virulence regulator PrfA. Microbiology 2008;154:3579–3589. [PubMed: 18957610]
60. Ripio M-T, Dominguez-Bernal G, Lara M, Suarez M, Vazquez-Boland J-A. A Gly145Ser substitution in the transcriptional activator PrfA causes constitutive overexpression of virulence factors in *Listeria monocytogenes*. J. Bacteriol 1997;179:1533–1540. [PubMed: 9045810]
61. Shetron-Rama LM, et al. Isolation of *Listeria monocytogenes* mutants with high-level *in vitro* expression of host cytosol-induced gene products. Mol. Microbiol 2003;48:1537–1551. [PubMed: 12791137]
62. Vega Y, et al. New *Listeria monocytogenes prfA** mutants, transcriptional properties of PrfA* proteins and structure–function of the virulence regulator PrfA. Mol. Microbiol 2004;52:1553–1565. [PubMed: 15186408]
63. Wong KK, Freitag NE. A novel mutation within the central *Listeria monocytogenes* regulator PrfA that results in constitutive expression of virulence gene products. J. Bacteriol 2004;186:6265–6276. [PubMed: 15342597]
64. Monk IR, Gahan CG, Hill C. Tools for functional postgenomic analysis of *Listeria monocytogenes*. Appl. Environ. Microbiol 2008;74:3921–3934. [PubMed: 18441118]
65. Mauder N, et al. Species-specific differences in the activity of PrfA, the key regulator of listerial virulence genes. J. Bacteriol 2006;188:7941–7956. [PubMed: 16980455]
66. 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]
67. Gray MJ, Freitag NE, Boor KJ. How the bacterial pathogen *Listeria monocytogenes* mediates the switch from environmental Dr. Jekyll to pathogenic Mr. Hyde. Infect. Immun 2006;74:2505–2512. [PubMed: 16622185]
68. Ollinger J, Bowen B, Wiedmann M, Boor KJ, Bergholz TM. *Listeria monocytogenes* σ^B modulates PrfA-mediated virulence factor expression. Infect. Immun 2009;77:2113–2124. [PubMed: 19255187]
69. Ollinger J, Wiedmann M, Boor KJ. σ^B- and PrfA-dependent transcription of genes previously classified as putative constituents of the *Listeria monocytogenes* PrfA regulon. Foodborne Pathog. Dis 2008;5:281–293. [PubMed: 18564909]
70. Toledo-Arana A, et al. The *Listeria* transcriptional landscape from saprophytism to virulence. Nature 2009;459:950–956. [PubMed: 19448609]
71. Mueller KJ, Freitag NE. Pleiotropic enhancement of bacterial pathogenesis resulting from the constitutive activation of the *Listeria monocytogenes* regulatory factor PrfA. Infect. Immun 2005;73:1917–1926. [PubMed: 15784531]
72. Ripio MT, et al. Transcriptional activation of virulence genes in wild-type strains of *Listeria monocytogenes* in response to a change in the extracellular medium composition. Res. Microbiol 1996;147:371–384. [PubMed: 8763623]
73. Marr AK, et al. Overexpression of PrfA leads to growth inhibition of *Listeria monocytogenes* in glucose-containing culture media by interfering with glucose uptake. J. Bacteriol 2006;188:3887–3901. [PubMed: 16707681]
74. Brockstedt DG, Dubensky TW. Promises and challenges for the development of *Listeria monocytogenes*-based immunotherapies. Expert Rev. Vaccines 2008;7:1069–1084. [PubMed: 18767955]

75. Schoen C, et al. *Listeria monocytogenes* as novel carrier system for the development of live vaccines. Int. J. Med. Microbiol 2008;298:45–58. [PubMed: 17936682]

76. Shen H, et al. Recombinant *Listeria monocytogenes* as a live vaccine vehicle for the induction of protective anti-viral cell-mediated immunity. Proc. Natl Acad. Sci. USA 1995;92:3987–3991. [PubMed: 7732018]

77. Wood LM, Guirnalda PD, Seavey MM, Paterson Y. Cancer immunotherapy using *Listeria monocytogenes* and listerial virulence factors. Immunol. Res 2008;42:233–245. [PubMed: 19018479]

78. Bruhn KW, Craft N, Miller JF. *Listeria* as a vaccine vector. Microbes Infect 2007;9:1226–1235. [PubMed: 17719258]

79. Lauer P, et al. Constitutive activation of the PrfA regulon enhances the potency of vaccines based on live-attenuated and killed but metabolically active *Listeria monocytogenes* strains. Infect. Immun 2008;76:3742–3753. [PubMed: 18541651]

80. Yan L, et al. Selected *prfA** mutations in recombinant attenuated *Listeria monocytogenes* strains augment expression of foreign immunogens and enhance vaccine-elicited humoral and cellular immune responses. Infect. Immun 2008;76:3439–3450. [PubMed: 18474644]

81. Tilney LG, Portnoy DA. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. J. Cell Biol 1989;109:1597–1608. [PubMed: 2507553]

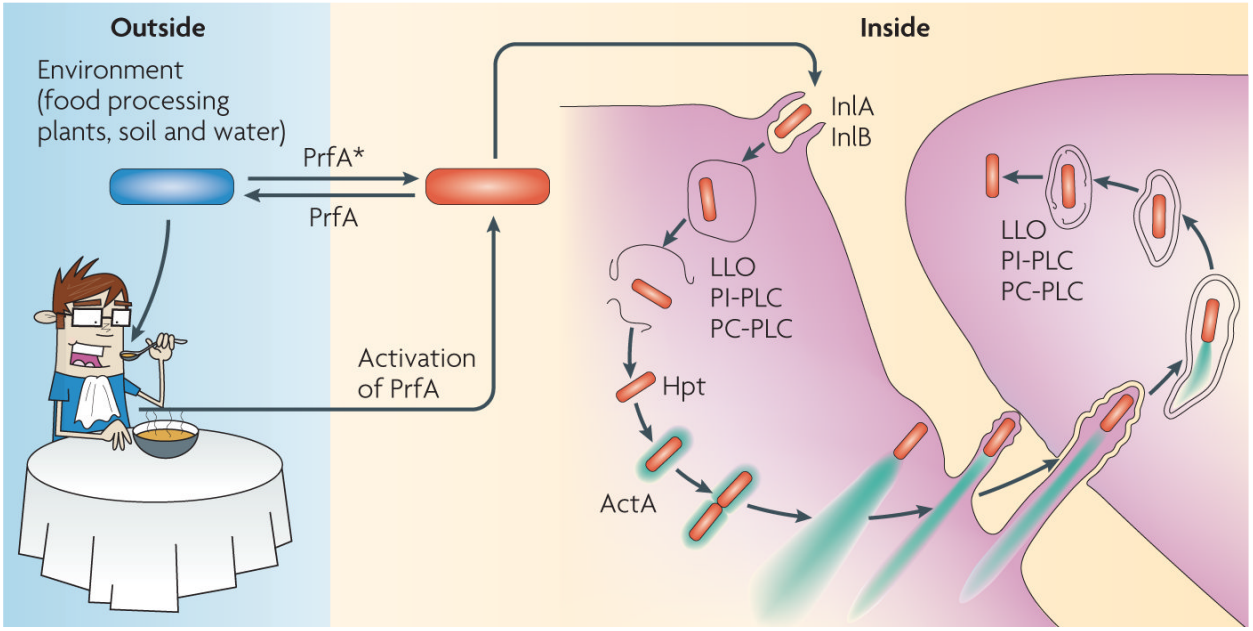

Figure 1. From saprophyte to intracellular pathogen

Listeria monocytogenes survives in a diverse array of environments, in habitats that include soil and water as well as food processing facilities. Central to the switch between life outside and life inside mammalian hosts is the transcriptional activator PrfA, which regulates the expression of many gene products that are required for bacterial virulence. Outside a host cell, PrfA exists in a low-activity state, with correspondingly low levels of virulence gene expression. Once inside the host, PrfA becomes activated (PrfA*) and induces the expression of gene products that are needed for host cell invasion (internalins InlA and InlB), phagosome lysis (listeriolysin O (LLO), phosphatidylinositol-specific phospholipase C (PI-PLC) and phosphatidylcholine (PC)-PLC), intracellular growth (hexose-6-phosphate transporter (Hpt)), and cell-to-cell spread (actin assembly-inducing protein (ActA); actin polymerization is shown in turquoise). The intracellular life cycle is modified, with permission, from REF. 81© (1989) Rockefeller University Press.

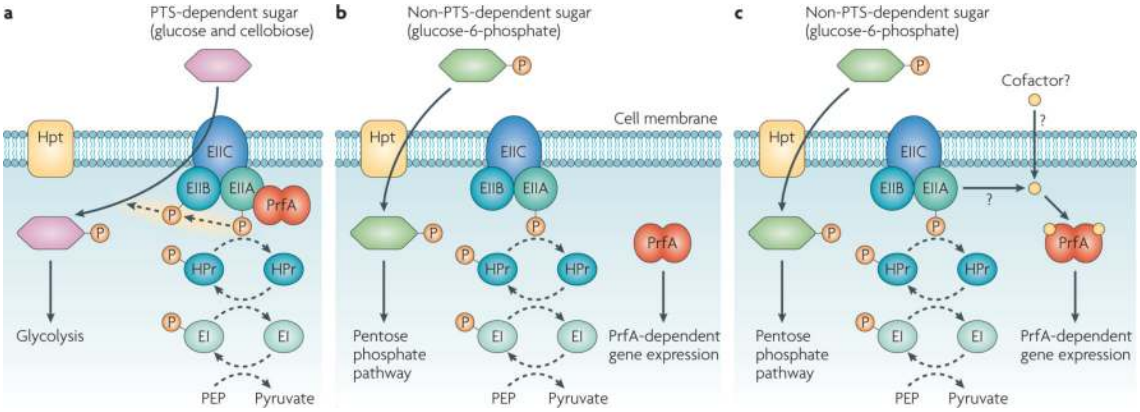

Figure 2. A model depicting the influence of carbon transport and metabolism on PrfA-dependent gene expression

The phosphoenol pyruvate (PEP) transport system (PTS) is a multiprotein phosphorelay system that couples the transport of sugars across the bacterial cell membrane with their simultaneous phosphorylation[54]. The PTS is composed of three distinct proteins: enzyme I (EI), histidine protein (HPr) and enzyme II (EII). A separate and distinct transporter, Hpt, mediates the transport of hexose phosphates, such as glucose-6-phosphate. **a** | In the presence of PTS-dependent sugars, EI (which autophosphorylates using the phosphoryl group from PEP) transfers a phosphoryl group to HPr, which then transfers it to the A domains of the various substrate-specific transporters or EIIs. During sugar transport, the phosphoryl group of EIIA is rapidly transferred to the EIIB domain and, from there, to the incoming carbohydrate as it passes through the membrane. EIIA therefore exists primarily in a non-phosphorylated state during active PTS sugar transport, and it is this form of EIIA that is postulated to sequester PrfA and inhibit its activity. **b** | In the presence of non-PTS-dependent sugars, such as glucose-6-phosphate, transport occurs through an alternative transporter such as Hpt. The EIIA component of PTS remains phosphor-ylated and is unable to sequester PrfA in this state. PrfA is released and can directly activate target promoters. **c** | Alternatively, PrfA that is released from EIIA may subsequently require the additional stimulus of an activating signal or cofactor to fully induce the expression of PrfA-dependent promoters.

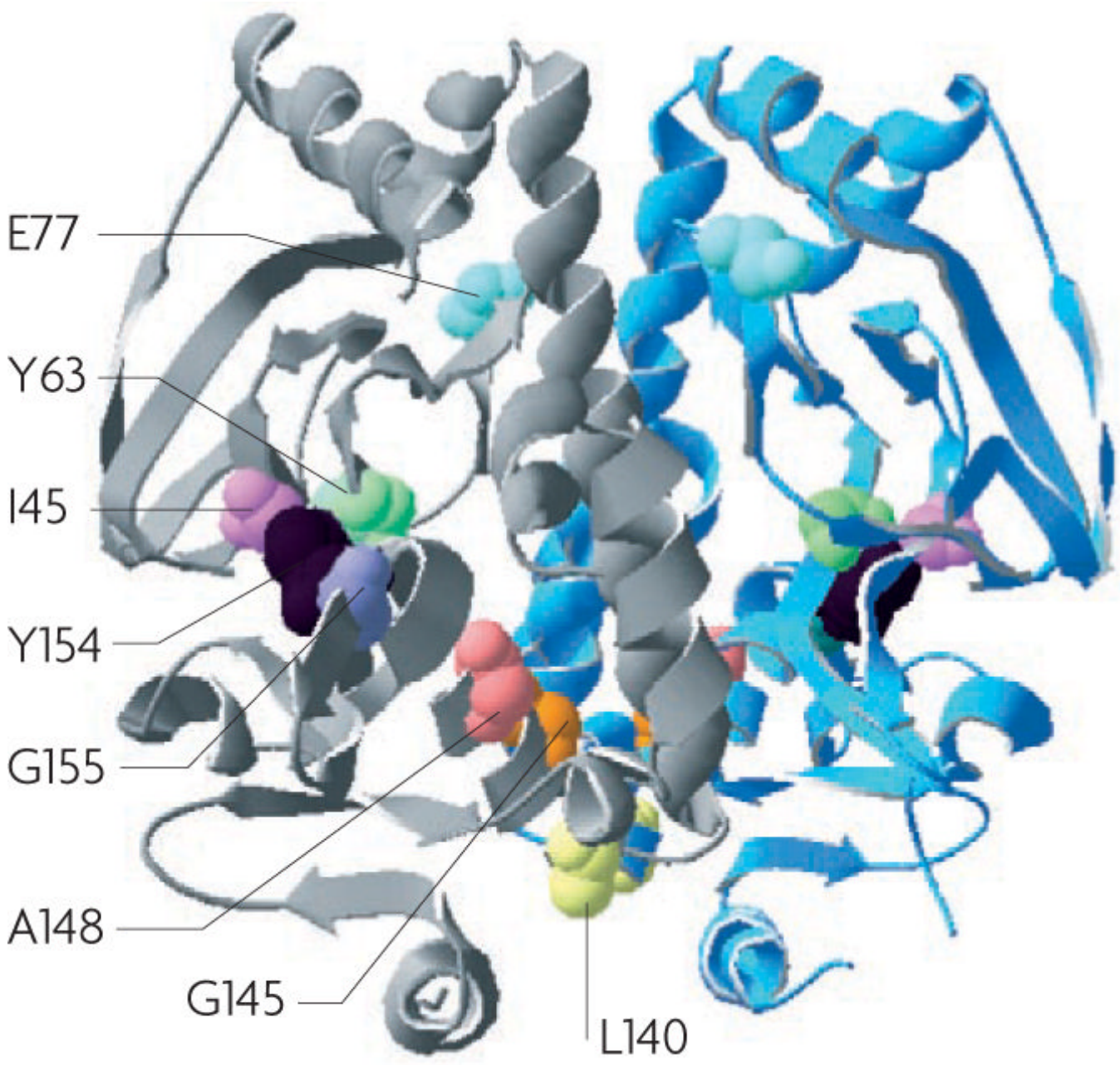

Figure 3. The location of mutations that result in the constitutive activation of PrfA

The crystal structure of a PrfA dimer (monomers are shown in grey and in light blue). The locations of the residues that are substituted in the PrfA* mutations described in the literature are as follows[8,58,59,64]: E77K, shown in light blue; Y63C, shown in light green; I45S, shown in pink; G155S, shown in purple; A148T, shown in salmon; G145S, G145R or G145C, shown in orange; L140F, shown in yellow. The Y154C mutation shown in black is unique in that it locks the protein into an activity state that enhances *in vitro* gene expression but does not provide full activation of PrfA-dependent gene expression *in vivo*58. Figure is modified, with permission, from REF. 59 © (2008) Society for General Microbiology.

Table 1

Listeria monocytogenes gene products that are directly regulated by PrfA

Gene name	Protein product	Function
hly	Listeriolysin O (LLO)	A pore-forming cytolysin that is required for phagosome lysis
plcA	Phosphatidylinositol-specific phospholipase C (PI-PLC)	Aids in phagosome lysis
plcB	Phosphatidylcholine phospholipase C (PC-PLC)	A broad substrate specificity phospholipase that aids in phagosome lysis
mpl	Mpl	A zinc metalloprotease that processes the PC-PLC precursor to its mature form
actA	Actin assembly-inducing ptotein (ActA)	Stimulates actin-based intracellular bacterial motility
hpt	Hexose phosphate transporter (Hpt)	Required for optimal intracellular bacterial growth
inlA	Internalin A (InlA)	Contributes to host cell invasion
inlB	Internalin B (InlB)	Contributes to host cell invasion
inlC	Internalin C (InlC)	Contributes to bacterial virulence; exact role unknown
prfA	Positive regulatory factor A (PrfA)	Required for the expression of *L. monocytogenes* virulence factors