The Journal of Microbiology, February 2005, p.110-117
Vol. 43, special issue (No. S)
Copyright © 2005, The Microbiological Society of Korea

# *Salmonella* Invasion Gene Regulation: A Story of Environmental Awareness

**Bradley D. Jones***

*Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242-1109, USA*

(Accepted November 9, 2004)

***Salmonella enterica* serovar Typhimurium causes human gastroenteritis and a systemic typhoid-like infection in mice. A critical virulence determinant of *Salmonella* is the ability to invade mammalian cells. The expression of genes required for invasion is tightly regulated by environmental conditions and a variety of regulatory genes. The *hilA* regulator encodes an OmpR/ToxR family transcriptional regulator that activates the expression of invasion genes in response to both environmental and genetic regulatory factors. Work from several laboratories has highlighted that regulation of *hilA* expression is a key point for controlling expression of the invasive phenotype. A number of positive regulators of *hilA* expression have been identified including *csrAB*, *sirA/barA*, *pstS*, *hilC/sirC/sprA*, *fis*, and *hilD*. HilD, an AraC/XylS type transcriptional regulator, is of particular importance as a mutation in *hilD* results in a 14-fold decrease in chromosomal *hilA*::Tn*5lacZY-080* expression and a 53-fold decrease in invasion of HEp-2 cells. It is believed that HilD directly regulates *hilA* expression as it has been shown to bind to *hilA* promoter sequences. In addition, our research group, and others, have identified genes (*hilE*, *hha*, *pag*, and *lon*) that negatively affect *hilA* transcription. HilE appears to be an important *Salmonella*-specific regulator that plays a critical role in inactivating *hilA* expression. Recent work in our lab has been directed at understanding how environmental signals that affect *hilA* expression may be processed through a *hilE* pathway to modulate expression of *hilA* and the invasive phenotype. The current understanding of this complex regulatory system is reviewed.**

*Key words*: *Salmonella* invasion, gene regulation, *hilA*, *hilE*, *hilD*

Pathogenic *Salmonella* species are an important cause of infectious diseases throughout the world. The organisms are transmitted via the fecal-oral route and cause human infections ranging from self-limiting gastroenteritis to typhoid fever. The strict human pathogen, *Salmonella enterica* serovar Typhi, causes typhoid fever, a systemic disease, which results in 16 million illnesses and 600,000 deaths worldwide each year (WHO, 1997). Transmission of this disease within the human population is generally a result of poor sanitation of water and food supplies in developing nations. Efforts to control disease transmission include improved sanitation practices and antibiotic treatment. However, infections with antibiotic resistant *Salmonella* species have surfaced, posing a greater risk to human populations in endemic areas (WHO, 1997). *S. enterica* serovars Enteritidis and Typhimurium cause the majority of human gastroenteritis infections: a reported 40,000 cases of salmonellosis in the U.S. each year. These broad host-range *Salmonella* serovars are prevalent within warm-blooded animal populations that make up the human food supply and bacterial transmission generally results from consumption of raw or undercooked food products (Darwin and Miller, 1999).

Efforts to understand the mechanisms by which *Salmonella* causes disease have lead to the development of tissue culture cell infection systems and animal models of infection. The use of these biological tools has been instrumental in characterizing many virulence factors necessary for *Salmonella* pathogenesis. For example, infection of epithelial and macrophage tissue culture cell lines has helped to identify and characterize the genetic elements necessary for invasion and intracellular survival of *Salmonella*. In addition, oral infection and intestinal ligated loop studies in murine models have allowed the investigation of virulence factors that mediate systemic pathogenesis reminiscent of typhoid fever, while rabbit and bovine models have provided valuable information about mechanisms of virulence that result in localized gastroenteritis (Wallis and Galyov, 2000). Thus, in vitro and in vivo studies have provided much information regarding the virulence factors required to mediate *Salmonella* disease.

* To whom correspondence should be addressed.
(Tel) 1-319-353-5457; (Fax) 1-319-335-9006
(E-mail) bradley-jones@uiowa.edu

One important *Salmonella* virulence trait is the ability to adhere to host intestinal tissue, which is mediated by several fimbrial types in different serovars of *Salmonella*. These include type I fimbriae, long polar fimbriae, plasmid-encoded fimbriae, and thin aggregative fimbriae (Darwin and Miller, 1999). Recent investigations have revealed the importance of specific amino acids within the adhesin molecule of type I fimbriae for mediating *Salmonella* binding and biofilm formation on tissue culture epithelial cells and mouse intestinal tissue (Boddicker *et al.*, 2002). Interestingly, long-polar fimbriae have been shown to mediate binding to Peyers patches overlying lymphoid follicles within the intestine (Darwin and Miller, 1999). This may be important, as serovar Typhimurium has been observed to preferentially invade and destroy M cells of Peyers patches in the murine model of *Salmonella* infection (Penheiter *et al.*, 1997).

The bacteria initiate invasion by expressing a type III secretion system that mediates injection of effector proteins from the bacteria into the cytoplasm of the eukaryotic cell. The effector proteins mediate actin cytoskeleton rearrangements, in the form of large membrane ruffles, which engulf the bacteria into the host cell (Darwin and Miller, 1999). Following invasion of intestinal cells, the subsequent destruction of M cells and enterocytes allows the bacteria to come into contact with macrophages residing in the underlying tissue. There, the secreted effector protein, SipB, appears to be involved in inducing cytokine signaling and apoptosis within macrophages, which may initially be important for bacterial survival and recruitment of additional phagocytes to facilitate systemic spread of the bacteria (Monack *et al.*, 2001). Importantly, *Salmonella* secrete a different set of effector proteins, through a second type III secretion system, that allows the bacteria to survive and replicate within macrophages at systemic sites of infection (Hensel, 2000).

Clearly, *Salmonella* pathogenesis is a complex phenomenon requiring the coordinate regulation of many bacterial virulence factors utilized at different anatomical sites within the animal host. Investigations in our laboratory and others have focused on the regulation of genes necessary to invade host cells, since these genes are required for the initial invasion of the intestinal epithelium that is essential for *Salmonella* to cause localized gastroenteritis, as well as systemic disease. Initial observations indicated that in vitro growth conditions, including bacterial growth state, osmolarity, and oxygen levels, affected the ability of *Salmonella* to invade tissue culture cells (Ernst *et al.*, 1990; Galán and Curtiss III, 1990;Lee and Falkow, 1990; McBeth and Lee, 1993). This suggested that *Salmonella* responds to a combination of environmental cues that allow repression of the invasive phenotype until the bacteria sense they are in the appropriate host environment to initiate invasion. Therefore, much effort has been expended to identify genetic elements within *Salmonella* that allow the bacteria to respond to environmental cues that stimulate or repress invasion.

***The HilA regulator***

The *hilA* gene (hyper-invasive locus A) was first identified as a locus that renders the bacteria non-responsive to high-oxygen repression of invasion when overexpressed (Lee *et al.*, 1992). The *hilA* gene is located in *Salmonella* pathogenicity island I (SPI-1), depicted in Fig. 1, which encodes structural components, chaperones, and secreted effectors of the type III secretion system necessary for invasion (Bajaj *et al.*, 1995;Darwin and Miller, 1999). Expression of *hilA* appears to be crucial for the invasive phenotype, as mutations in *hilA* decrease invasion ~150-fold and mouse virulence ~60-fold (Bajaj *et al.*, 1995; Penheiter *et al.*, 1997). The amino terminus of *hilA* is homologous to the DNA binding domain of the OmpR/ToxR family of transcriptional activators, which suggests that *hilA* is a regulatory element in the invasion process. Subsequently, the effects of *hilA* mutations on the expression of invasion genes located on SPI-1 were measured. An approximate 10-fold decrease in the expression of genes located within the *prg*, *inv/spa*, and *sip* operons was observed in strains containing a mutation in *hilA*, supporting the idea that HilA activates invasion gene expression (Bajaj *et al.*, 1995; Bajaj *et al.*, 1996). Additional studies demonstrated that HilA binds to the promoters of the *prg* and *inv/spa* operons at consensus sites termed "HilA boxes" to promote their transcription (Lucas *et al.*, 2000). While HilA directly activates the *prg* and *inv/spa* transcriptional units, HilA indirectly activates the the *sip* operon (Darwin and Miller, 1999). Apparently, HilA activates the expression of *invF* and *sicA,* whose genes respectively encode an AraC/XylS transcriptional activator and a chaperone, that together activate transcription of the *sip* operon. InvF and SicA also activate transcription of *sopB/sigD,* and *sopE,* found elsewhere on the chromosome, that encode secreted effector proteins that enhance invasion (Ahmer *et al.*, 1999; Darwin and Miller, 1999; Eichelberg and Galán, 1999; Darwin and Miller, 2000; Darwin and Miller, 2001). Thus, HilA is a central tran-

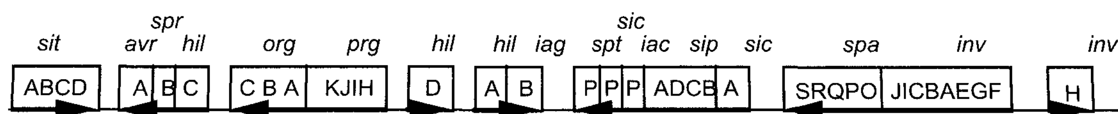


**Fig. 1.** *Salmonella* Pathogenicity Island I (SPI-1). Gene map of *Salmonella* invasion gene located at centisome 63. Arrows indicate direction of transcription. Genes encode structural components of the secretion machinery, effector proteins, chaperone proteins, and regulators as described in the text.

scriptional regulator of the invasion genes found on SPI-1, as well as invasion genes found at other chromosomal loci.

***Environmental regulation of invasion***

Expression of *lacZY* reporters fused to *invF, invA, sipA, sipC, prgH, prgK,* or *orgA* was reduced after bacterial growth in conditions known to be unfavorable for invasion (Galán and Curtiss III, 1990; Jones and Falkow, 1994; Bajaj *et al.*, 1996). Reduction in invasion gene expression was comparable to the reduction observed in a *hilA* mutant background. These findings suggested that HilA coordinately regulates the transcription of invasion genes in response to environmental cues. In support of this hypothesis, *hilA*, expressed from a constitutive *neo* promoter, almost completely abrogates the repressing effects of environmental conditions on downstream invasion gene expression (Bajaj *et al.*, 1996). Since constitutive *hilA* expression is able to override repressing environmental signals, modulation of *hilA* expression is a likely way for the bacteria to channel environmental cues into the regulation of invasion gene expression. Therefore, the effect of environmental growth conditions on *hilA-lacZY* expression has been examined. Optimal expression of *hilA* occurs in low oxygen, high osmolarity, exponential phase growth, as well as slightly alkaline pH, the same conditions that induce downstream invasion genes (Bajaj *et al.*, 1995). Thus, modulation of *hilA* expression appears to be a central mechanism for regulating invasion gene expression in response to environmental cues. Of note, it has been demonstrated that HilA does not auto-regulate *hilA* expression (Bajaj *et al.*, 1995).

Genetic approaches have been employed to detemine how environmental signals modulate *hilA* expression. A mutation in the *sirA* gene resulted in ~6 -fold decrease in *prgH* expression, as well as ~6 -fold decrease in *hilA* expression, and a corresponding decrease in invasion of tissue culture cells. Plasmid-encoded *sirA* restored *hilA* expression and invasion (Johnston *et al.*, 1996), indicating that SirA modulates *hilA* transcription. Other work found that a *sirA* mutation decreases the ability of *Salmonella* to cause symptoms of gastroenteritis, including PMN influx and fluid secretion, in the bovine ligated loop model (Ahmer *et al.*, 1999). SirA is a member of the FixJ family of transcriptional activators that has 96% identity to the *uvrY* gene product from *E. coli* and is predicted to be a response regulator of a two-component regulatory system (Johnston *et al.*, 1996). UvrY is an important global regulator, as a mutation in *uvrY* affects the expression of over 100 genes in *E. coli*, including flagellar genes and the *rpoS*-dependent stationary phase regulon (Oshima *et al.*, 2002). Interestingly, homologues of *sirA* and *uvrY*, that are associated with the regulation of pathogenesis genes, exist in several other gram negative bacteria, including *gacA* of *Pseudomonas* species, *varA* of *Vibrio cholerae*, and *letA* of *Legionella pneumophila* (Reimmann *et al.*, 1997; Wong *et al.*, 1998; Blumer and Haas, 2000; Hammer *et al.*, 2002). BarA has been identified as a partner sensor kinase of SirA (Altier *et al.*, 2000; Pernestig *et al.*, 2001). BarA is a member of the sensor histidine kinase family of proteins, with a predicted inner-membrane spanning region and periplasmic sensing domain (Nagasawa *et al.*, 1992; Altier *et al.*, 2000). Like SirA, BarA is found in other bacterial genera and has been shown to act as a virulence factor for uropathogenic *E. coli* (Zhang and Normark, 1996). The effects of a *barA* mutation are similar to those of a *sirA* mutation on *hilA* expression and invasion of tissue culture cells, and a non-polar *barA* mutation can be complemented by plasmid encoded *barA*.

***Transcriptional activators of hilA***

Several research groups have searched for genes that activate *hilA* expression. Two *Salmonella* specific SPI-1 encoded genes have been implicated in the regulation of *hilA* expression (Schechter *et al.*, 1999). In addition, the *rtsA*/*rtsB* genes have also been shown to induce expression of *hilA*. The *hilC* and *hilD* genes were identified when it was discovered that a *hilA-lacZY* plasmid reporter could not be expressed within *Salmonella* when the entire SPI-1 locus was deleted from the chromosome. Subcloning of SPI-1 genes identified *hilC* and *hilD* as activators of *hilA* expression (Schechter *et al.*, 1999). Both HilC and HilD are predicted to be members of the AraC/XylS family of transcriptional activators, based on homology within their C-terminal domains that contain a characteristic helix-turn-helix DNA binding motif (Eichelberg *et al.*, 1999; Rakeman *et al.*, 1999; Schechter *et al.*, 1999). A mutation in HilD results in an approximately 10-fold decrease in *hilA* expression and 53-fold decrease in invasion of cultured epithelial cells (Schechter *et al.*, 1999). However, a mutation in *hilC* (*sirC/sprA*) results in a modest 50% decrease in *hilA* expression, and only a slight (20% to ~3-fold) decrease in invasion (Eichelberg *et al.*, 1999; Rakeman *et al.*, 1999; Schechter *et al.*, 1999; Lucas and Lee, 2001). Though, HilC appears to be less critical for *hilA* expression and invasion than HilD in these experiments, expression of *hilC* from a plasmid compensated for a mutation in *hilD* to promote high levels of *hilA* expression, suggesting that HilC and HilD are functionally redundant (Schechter *et al.*, 1999; Lucas and Lee, 2001). Both proteins were shown to bind specifically to the *hilA* promoter in gel mobility shift assays, and DNase I footprinting experiments indicated that HilD binds in two regions, from 231 to 179 and from 101 to 49 (Schechter and Lee, 2001). Initially, *hilC* and *hilD* were reported to be derepressors of invasion gene expression that would function by counteracting the action of negative regulatory elements at the *hilA* promoter (Schechter *et al.*, 1999). However, evidence from our laboratory indicates that HilD is a required activator of *hilA* expression,

necessary for contact and recruitment of RNA polymerase at the *hilA* promoter (Boddicker *et al.*, 2002). Similar to HilC and HilD, RtsA belongs to the AraC/XylS family of regulators, while RtsB is a helix-turn-helix DNA binding protein. RtsA increases invasion gene expression by increasing transcription of *hilA*, which is independent of HilC and HilD. Similar to a *hilC* mutant, a *rtsA* mutant decreases *hilA* expression ~2-fold. These data suggest that HilC and RtsA may activate *hilA* expression in response to a set of signals that are not present in laboratory conditions.

While HilA is an important regulator of invasion gene expression, other work indicates that a HilA-independent regulatory pathway for expression of invasion genes also exists. Overexpression of *hilC* (*sirC/sprA*) can overcome the requirement for HilA activation of the *inv* operon, and subsequent transcription of genes encoding secreted effector proteins (Eichelberg and Galán, 1999; Rakeman *et al.*, 1999). Some reports suggest that overexpression of *sirA* can also overcome the requirement for HilA activation of the *inv* operon (Eichelberg and Galán, 1999; Rakeman *et al.*, 1999). However, work from another group found that overexpression of *sirA* does not overcome the requirement for *hilA* activation (Ahmer *et al.*, 1999). Though it appears that HilA-independent pathways for transcription of type III secreted effectors proteins from the *inv* and *sic/sip* operons may exist, HilA is required for the expression of genes encoding the type III secretion apparatus from the *prg* operon (Rakeman *et al.*, 1999). Since mutations in *hilC* appear to have little or no effect on invasion gene expression or the invasive phenotype, an understanding of the role of HilC in pathogenesis will have to await future studies (Eichelberg *et al.*, 1999; Rakeman *et al.*, 1999; Schechter *et al.*, 1999).

### *Other invasion gene activators*

In addition to these possible regulatory pathways, another layer of invasion gene regulation has been discovered. The *csrA* and *csrB* genes have been implicated in controlling levels of *hilA* expression through modulation of *hilD* and *hilC* transcript levels. The CsrA protein destabilizes specific mRNA molecules by interactions at the ribosome-binding site. The *csrB* gene encodes an untranslated RNA that counteracts CsrA function by binding CsrA molecules to prevent degradation of mRNA (Yang *et al.*, 1996; Liu and Romeo, 1997). The *csrB* locus was identified by mutagenesis and found to have a mild affect on *hilA* transcription that did not attenuate invasion (Altier *et al.*, 2000). Since *csrB* counteracts the effect of CsrA, the effects of overexpression or deletion of *csrA* were also examined. Overexpression of *csrA* and, unexpectedly, deletion of *csrA* resulted in a ~10-fold defect in *hilA* expression and ~100-fold defect in invasion of epithelial cells (Altier *et al.*, 2000a, 2000b). Thus, CsrA appears to have both a positive and negative regulatory effect on invasion gene expression. The defect in *hilA* expression has been determined to be indirect. Subsequent experiments demonstrated that *hilC* and *hilD* transcripts, examined by Northern blot analysis, were undetectable in strains that had a deletion in *csrA* or overexpressed *csrA* from an arabinose-inducible promoter. The effect of overexpression or deletion of *csrA* appeared to be specific for *hilC* and *hilD*, as *phoP*, *barA*, and *sirA* transcripts were unaffected (Altier *et al.*, 2000a, 200b). Thus, the *csrA/csrB* regulatory system appears to play a role in maintaining appropriate levels of *hilD* and *hilC* transcripts for subsequent activation of *hilA* expression. Since it appears that the *csrA/csrB* system maintains relatively constitutive levels of *hilD* and *hilC* transcripts when the bacteria are grown in vitro, one can speculate that this layer of invasion gene regulation may tilt the balance toward repression or activation of invasion at in vivo sites that cannot be mimicked by in vitro assay conditions. Interestingly, a link between the *csrA/csrB* regulatory system and the *uvrY* (*sirA*)/*barA* two-component regulatory system has recently been discovered in *E. coli*. It appears that *csrA* is necessary to regulate the activity of UvrY, which in turn activates the expression of *csrB* (Suzuki *et al.*, 2002). Therefore, it is possible that the *csrA/csrB* system modulates the expression of invasion genes at several levels, in a *sirA* dependent (*hilA* via *hilC*) and *sirA*-independent (*hilD*) manner. Further studies in *Salmonella* are required to determine if the *csrA/csrB* system modulates the activity of SirA to affect the expression of invasion genes.

A *pstS* mutation resulted in a ~3-fold decrease in *hilA* expression that was complemented by expression of the *pstSCAB-phoU* operon on a plasmid (Lucas *et al.*, 2000). A mutation in *pstS* causes low intracellular phosphate ($P_i$) levels due to a defective inorganic phosphate transport system. In conditions of low $P_i$, or in the presence of a *pstS* mutation, PhoB becomes phosphorylated to activate expression of the Pho regulon (Wanner, 1996). It appears that PhoB mediates the effect on *hilA* expression due to a mutation in *pstS*, as the effects of the *pstS* mutation are lost in a *pstS/phoB* strain. Thus, it appears that phosphorylated-PhoB may decrease *hilA* expression in response to low $P_i$ levels. Currently, it has not been demonstrated that phosphate levels regulate invasion gene expression. It would be interesting to contrast the levels of wt *hilA* expression to those in a *phoB* mutant strain when *Salmonella* is grown in limiting phosphate conditions.

### *Negative regulators of invasion*

Genetic screens conducted in our laboratory have identified several genetic loci that are involved in negative regulation of *hilA* expression and invasion. The *hha* gene, common to *Salmonella* and other bacterial genera, as well as two *Salmonella* specific genes, *pag* and *hilE* have been identified by mutational analysis. In addition, studies conducted by Takaya et. al, have identified the Lon protease

as an important negative regulator of *hilA* expression (Takaya *et al.*, 2002). Mutations in *pag* caused ~5-fold increases in *hilA* expression. A mutation in the *lon* gene, that encodes Lon protease, resulted in ~10-fold increased *hilA*-expression, increased secretion of effector proteins, and ~10-fold increased invasion of epithelial cells. The effects of a *lon* mutation were complemented by expression of *lon* on a plasmid (Takaya *et al.*, 2002). It will be interesting to discover what protein(s) Lon degrades to modulate the expression of *hilA*. Since *hilD* transcription appears to be constitutive, one possible mechanism for Lon activity is that it degrades HilD in repressing environmental conditions to regulate *hilA* expression.

The *hha* gene encodes a small DNA binding protein, first identified by the ability of a gene bank clone carrying *hha* to repress *hilA-lacZY* expression. Overexpression of the *hha* gene from a plasmid decreases the expression of *hilA* ~7-fold, reduces invasion of epithelial cells by ~40-fold, and virtually eliminates the ability of *Salmonella* to invade and destroy Peyers patches in mouse ligated intestinal loops. Conversely, a mutation in *hha* caused ~3-fold increased *hilA* expression, and ~10-fold increased invasion of epithelial cells when the bacteria were grown in repressing conditions for invasion. *Salmonella* Hha is a small nucleoid-associated histone-like protein, that is 99% identical to *hha* from *E. coli*, and can function interchangeably in *E. coli* to repress the expression of the hemolysin operon. Importantly, Hha has been shown to bind to the *hilA* promoter (Fahlen *et al.*, 2001). Nucleoid-associated proteins can affect the supercoiling of DNA and also modify the expression of genes in response to environmental conditions (Travers and Muskhelishvili,

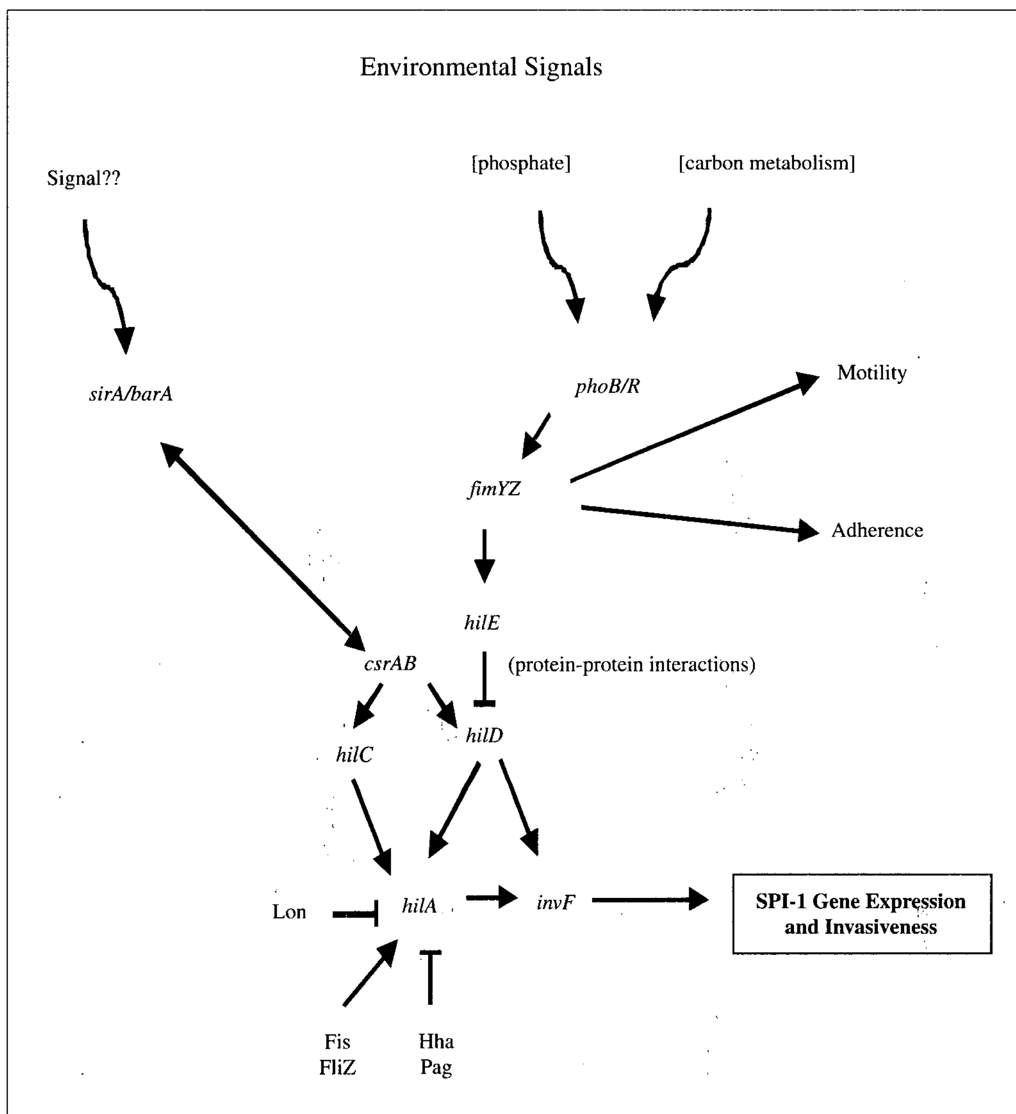


**Fig. 2.** Regulatory scheme of environmental signal processing to upregulate or downregulate *hilA* transcription and expression of the *Salmonella* invasive phenotype. Arrows represent the direction signal are transduced through the regulatory cascade. FimZ is involved in invasive signal processing as well as motility and adherence (via type 1 fimbriae) regulation.

1998). Thus, Hha may modify the architecture of the *hilA* promoter to negatively regulate its expression.

A mutation in the *hilE* gene causes a ~5-fold increase in *hilA* expression that results in a ~15-fold increase in invasion of epithelial cells when bacteria are grown in repressing conditions for invasion (Fahlen *et al.*, 2000; Baxter *et al.*, 2003). However, overexpression of *hilE* from a single-copy plasmid causes ~4-fold repression of *hilA* expression and ~19-fold decrease in invasion of epithelial cells. Efforts in our laboratory to demonstrate binding of purified HilE protein to the *hilA* promoter have failed, however, recent work demonstrated that the HilE protein interacts with HilD, using a bacterial two-hybrid assay (Baxter *et al.*, 2003). This finding suggests that HilE functions as a repressor by binding to HilD to prevent its activation of the *hilA* promoter. Recent work in our laboratory indicates that *hilE* expression may be a key point in processing signals that affect *hilA* transcription and expression of the invasive phenotype (Baxter and Jones, 2004). A screen for mutations that affect *hilE-lacZY* transcription identified the *fimYZ* genes as activators of *hilE* transcription. The *fimYZ* genes encode response-regulator type proteins that activate the expression of type I fimbriae in *Salmonella*, and repress the expression of flagellar genes and motility (Clegg and Hughes, 2002). However, no sensor kinase partner has yet been identified for the FimY/FimZ proteins. Since a mutation in *pstS* results in high-level phosphorylation of PhoB and decreases *hilA* expression, we reasoned that a *pstS* mutation might increase both *fimYZ* and *hilE* expression as an explanation for the effect on *hilA* transcription. Assays measuring the effect of a *pstS* mutation on both *fimZ-lacZY* and *hilE-lacZY* expression revealed increases of >20-fold and ~3-fold respectively. As an additional test, a mutation in *hilE* was found to abrogate repression of *hilA* by the *pstS* mutation (Baxter and Jones, unpublished). Collectively, these data indicate that the effects of a *pstS* mutation, which decrease *hilA* expression, are mediated through the activation of the negative regulators, *hilE* and *fimYZ*. These results indicate that at a *fimYZ*-dependent pathway modulates *hilE* transcription and invasion gene repression.

## Conclusion

Clearly the activation and repression of invasion genes in *Salmonella* is a complex phenomenon involving many layers of regulation. Current work in our laboratory and others has lead to a working model of invasion gene regulation (Fig. 2), which predicts that the effects of environmental growth conditions on invasion gene expression are mediated in several ways. The effects of certain environmental conditions, such as growth phase, oxygen, and osmolarity, may be mediated by small DNA binding proteins, such as Hha and Fis, whose functions can couple cellular physiology to the topology of the bacterial chromosome, altering DNA supercoiling and transcription. These factors clearly regulate the expression of *hilA*, but may also modulate invasion gene expression at other levels upstream and downstream of *hilA* in the regulatory network. It appears that certain environmental conditions, such as phosphate or extracellular cation limitations, may be specifically sensed and mediated by two-component phosphorelay systems, such as PhoR/PhoB, which can increase the expression of the *hilE* invasion repressor gene. Since multiple factors are involved in mediating invasion gene regulation, including specific sensing and signal transduction mechanisms, as well as modulation of DNA supercoiling due to the physiological state of the cell and small DNA binding proteins, it has been difficult to fully define the regulatory networks that mediate a particular environmental signal, such as osmolarity. However, it may be possible to abrogate the effects of certain environmental signals on the invasive phenotype, if they are mediated in a linear pathway by two-component regulatory systems. Ongoing work is aimed at unraveling the network of interactions that occur to activate or repress invasion gene expression, as well as to identify the signaling mechanisms that allow environmental cues to be processed. In addition, efforts are being made to identify other components of this complex regulatory system. While many studies have described invasion gene regulation in response to in vitro environmental cues, future studies should be aimed at correlating these finding with the expression of regulatory elements and invasion genes in vivo. This work should provide a better understanding of the ability of *Salmonella* to respond to environmental cues that signal the bacteria to invade host cells, which may lead to the discovery of novel therapeutic targets at this important step in *Salmonella* pathogenesis.

## References


Ahmer, B.M., R.J. van, P.R. Watson, T.S. Wallis, and F. Heffron. 1999. *Salmonella* SirA is a global regulator of genes mediating enteropathogenesis. *Mol. Microbiol.* 31, 971-982.

Altier, C., M. Suyemoto, and S.D. Lawhon. 2000a. Regulation of *Salmonella enterica* serovar Typhimurium invasion genes by *csrA*. *Infect Immun.* 68, 6790-6797.

Altier, C., M. Suyemoto, A.I. Ruiz, K.D. Burnham, and R. Maurer. 2000b. Characterization of two novel regulatory genes affecting *Salmonella* invasion gene expression. *Mol. Microbiol.* 35, 1872-1882.

Altier, C., M. Suyemoto, A.I. Ruiz, K.D. Burnham, and R. Maurer. 2000. Characterization of two novel regulatory genes affecting *Salmonella* invasion gene expression. *Mol. Microbiol.* 35, 635-646.

Bajaj, V., C. Hwang, and C.A. Lee. 1995. *hilA* is a novel *ompR/toxR* family member that activates the expression of *Salmonella typhimurium* invasion genes. *Mol. Microbiol.* 18, 715-727.

Bajaj, V., R.L. Lucas, C. Hwang, and C.A. Lee. 1996. Co-ordinate regulation of *Salmonella typhimurium* invasion genes by environmental and regulatory factors is mediated by control of *hilA*

expression. *Mol. Microbiol.* 22, 703-714.

Baxter, M.A., T.F. Fahlen, R.L. Wilson, and B.D. Jones. 2003. HilE interacts with HilD and negatively regulates *hilA* transcription and expression of the *Salmonella enterica* serovar Typhimurium invasive phenotype. *Infect. Immun.* 71, 1295-1305.

Baxter, M.A. and B.D. Jones. 2004. The *fimYZ* genes regulate *Salmonella* invasion, in addition to type 1 fimbrial expression and bacterial motility. *Infect Immun.* in press.

Blumer, C. and D. Haas. 2000. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Arch. Microbiol.* 173, 170-177.

Boddicker, J.D., N.A. Ledeboer, J. Jagnow, B.D. Jones, and S. Clegg. 2002. Differential binding to andbiofilm formation on, HEp-2 cells by Salmonella enterica SerovarTyphimurium is dependent upon allelic variation in the fimH geneof the fim gene cluster. *Mol. Microbiol.* 45, 1255-1265.

Clegg, S. and K.T. Hughes. 2002. FimZ is a molecular link between sticking and swimming in *Salmonella enterica* serovar Typhimurium. *J Bacteriol.* 184, 1209-1213.

Darwin, K.H. and V.L. Miller. 1999. InvF is required for expression of genes encoding proteins secreted by the SPI1 type III secretion apparatus in Salmonella typhimurium. *J Bacteriol.* 181, 4949-4954.

Darwin, K.H. and V.L. Miller. 1999. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. *Clin. Microbiol. Rev.* 12, 405-428.

Darwin, K.H. and V.L. Miller. 2000. The putative invasion protein chaperone SicA acts together with InvF to activate the expression of *Salmonella typhimurium* virulence genes. *Mol. Microbiol.* 35, 949-960.

Darwin, K.H. and V.L. Miller. 2001. Type III secretion chaperone-dependent regulation: activation of virulence genes by SicA and InvF in *Salmonella typhimurium. EMBO J.* 20, 1850-1862.

Eichelberg, K. and J.E. Galán. 1999. Differential regulation of *Salmonella typhimurium* type III secreted proteins by pathogenicity island 1 (SPI-1)-encoded transcriptional activators InvF and HilA. *Infect. Immun.* 67, 4099-4105.

Eichelberg, K., W.D. Hardt, and J.E. Galán. 1999. Characterization of SprA, an AraC-like transcriptional regulator encoded within the *Salmonella typhimurium* pathogenicity island 1. *Mol. Microbiol.* 33, 139-152.

Ernst, R.K., D.M. Dombroski, and J.M. Merrick. 1990. Anaerobiosis, type 1 fimbriae, and growth phase are factors that affect invasion of HEp-2 cells by *Salmonella typhimurium. Infect. Immun.* 58, 2014-2016.

Fahlen, T.F., N. Mathur, and B.D. Jones. 2000. Identification and characterization of mutants with increased expression of *hilA*, the invasion gene transcriptional activator of *Salmonella typhimurium. FEMS Immunol. Med. Microbiol.* 28, 25-35.

Fahlen, T.F., R.W. Wilson, J.D. Boddicker, and B.D. Jones. 2001. Hha is a negative modulator of *hilA* transcription, the *Salmonella typhimurium* invasion gene transcriptional activator. *J Bacteriol.* 183, 6620-6629.

Galán, J.E. and R. Curtiss III. 1990. Expression of *Salmonella typhimurium* genes required for invasion is regulated by changes in DNA supercoiling. *Infect. Immun.* 58, 1879-1885.

Hammer, B.K., E.S. Tateda, and M.S. Swanson. 2002. A two-component regulator induces the transmission phenotype of stationary-phase *Legionella pneumophila. Mol. Microbiol.* 44, 107-118.

Hensel, M. 2000. *Salmonella* pathogenicity island 2. *Mol. Microbiol.* 36, 1015-1023.

Johnston, C., D.A. Pegues, C.J. Hueck, A. Lee, and S.I. Miller. 1996. Transcriptional activation of *Salmonella typhimurium* invasion genes by a member of the phosphorylated response-regulator superfamily. *Mol. Microbiol.* 22, 715-727.

Jones, B.D. and S. Falkow. 1994. Identification and characterization of a *Salmonella typhimurium* oxygen-regulated gene required for bacterial internalization. *Infect. Immun.* 62, 3745-3752.

Lee, C.A. and S. Falkow. 1990. The ability of *Salmonella* to enter mammalian cells is affected by bacterial growth state. *Proc. Natl. Acad. Sci. USA.* 87, 4304-4308.

Lee, C.A., B.D. Jones, and S. Falkow. 1992. Identification of a *Salmonella typhimurium* invasion locus by selection for hyperinvasive mutants. *Proc. Natl. Acad. Sci. USA.* 89, 1847-1851.

Liu, M.Y. and T. Romeo. 1997. The global regulator CsrA of *Escherichia coli* is a specific mRNA-binding protein. *J Bacteriol.* 179, 4639-4642.

Lucas, R.L., C.P. Lostroh, C.C. DiRusso, M.P. Spector, B.L. Wanner, and C.A. Lee. 2000. Multiple factors independently regulate *hilA* and invasion gene expression in *Salmonella enterica* serovar typhimurium. *J Bacteriol.* 182, 1872-1882.

Lucas, R.L. and C.A. Lee. 2001. Roles of *hilC* and *hilD* in Regulation of *hilA* Expression in *Salmonella enterica* Serovar Typhimurium. *J Bacteriol.* 183, 2733-2745.

McBeth, K.J. and C.A. Lee. 1993. Prolonged inhibition of bacterial protein synthesis abolishes *Salmonella* invasion. *Infect. Immun.* 61, 1544-1546.

Monack, D.M., W.W. Navarre, and S. Falkow. 2001. *Salmonella*-induced macrophage death: the role of caspase-1 in death and inflammation. *Microbes Infect.* 3, 1201-1212.

Nagasawa, S., S. Tokishita, H. Aiba, and T. Mizuno. 1992. A novel sensor-regulator protein that belongs to the homologous family of signal-transduction proteins involved in adaptive responses in *Escherichia coli. Mol. Microbiol.* 6, 799-807.

Oshima, T., H. Aiba, Y. Masuda, S. Kanaya, M. Sugiura, B.L. Wanner, H. Mori, and T. Mizuno. 2002. Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12. *Mol Microbiol.* 46, 281-291.

Penheiter, K.L., N. Mathur, D. Giles, T. Fahlen, and B.D. Jones. 1997. Non-invasive *Salmonella typhimurium* mutants are avirulent because of an inability to enter and destroy M cells of ileal Peyer's patches. *Mol. Microbiol.* 24, 697-709.

Pernestig, A.K., O. Melefors, and D. Georgellis. 2001. Identification of UvrY as the cognate response regulator for the BarA sensor kinase in *Escherichia coli. J Biol. Chem.* 276, 225-231.

Rakeman, J.L., H.R. Bonifield, and S.I. Miller. 1999. A HilA-independent pathway to *Salmonella typhimurium* invasion gene transcription. *J Bacteriol.* 181, 3096-3104.

Reimmann, C., M. Beyeler, A. Latifi, H. Winteler, M. Foglino, A. Lazdunski, and D. Haas. 1997. The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer N-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. *Mol. Microbiol.* 24, 309-319.

Schechter, L.M., S.M. Damrauer, and C.A. Lee. 1999. Two AraC/XylS family members can independently counteract the effect of repressing sequences upstream of the *hilA* promoter. *Mol. Microbiol.* 32, 629-642.

Schechter, L.M. and C.A. Lee. 2001. AraC/XylS family members,

HilC and HilD, directly bind and derepress the *Salmonella typhimurium hilA* promoter. *Mol. Microbiol.* 40, 1289-1299.

Suzuki, K., X. Wang, T. Weilbacher, A.K. Pernestig, O. Melefors, D. Georgellis, P. Babitzke, and T. Romeo. 2002. Regulatory circuitry of the CsrA/CsrB and BarA/UvrY systems of *Escherichia coli. J Bacteriol.* 184, 5130-5140.

Takaya, A., T. Tomoyasu, A. Tokumitsu, M. Morioka, and T. Yamamoto. 2002. The ATP-dependent lon protease of *Salmonella enterica* serovar Typhimurium regulates invasion and expression of genes carried on *Salmonella* pathogenicity island 1. *J Bacteriol.* 184, 224-232.

Travers, A. and G. Muskhelishvili. 1998. DNA microloops and microdomains: a general mechanism for transcription activation by torsional transmission. *J Mol. Biol.* 279, 1027-1043.

Wallis, T.S. and E.E. Galyov. 2000. Molecular basis of *Salmonella*-induced enteritis. *Mol. Microbiol.* 36, 997-1005.

Wanner, B.L. 1996. Phosphorus assimilation and control of the phosphate regulon, p. 1357-1381. *In* Neidhardt, F.C., et al (eds.) *Esherichia coli* and *Salmonella* : Cellular and Molecular Biology. vol. 1, ASM press, Washington, D.C.

WHO Fact Sheet. 1997, World Health Organization.

Wong, S.M., P.A. Carroll, L.G. Rahme, F.M. Ausubel, and S.B. Calderwood. 1998. Modulation of expression of the ToxR regulon in *Vibrio cholerae* by a member of the two-component family of response regulators. *Infect. Immun.* 66, 5854-5861.

Yang, H., M.Y. Liu, and T. Romeo. 1996. Coordinate genetic regulation of glycogen catabolism and biosynthesis in *Escherichia coli* via the CsrA gene product. *J Bacteriol.* 178, 1012-1017.

Zhang, J.P. and S. Normark. 1996. Induction of gene expression in *Escherichia coli* after pilus-mediated adherence. *Science.* 273, 1234-1236.