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Adaptation to the host environment: regulation of the SPI1 type III secretion system in *Salmonella enterica* serovar Typhimurium

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Salmonella enterica invades the intestinal epithelium of the host using a type III secretion system encoded on *Salmonella* pathogenicity island 1 (SPI1). The bacteria integrate environmental signals from a variety of global regulatory systems to precisely induce transcription of SPI1. The regulatory circuit converges on expression of HilA, which directly regulates transcription of the SPI1 apparatus genes. Transcription of *hilA* is controlled by a complex feed-forward loop. Regulatory signals feed into the system through post-transcriptional and post-translational control of HilD, which in turn activates HilC and RtsA. These three regulators act in concert to control *hilA* transcription. The system acts as a switch, ensuring that SPI1 is fully on at the appropriate time.

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Introduction

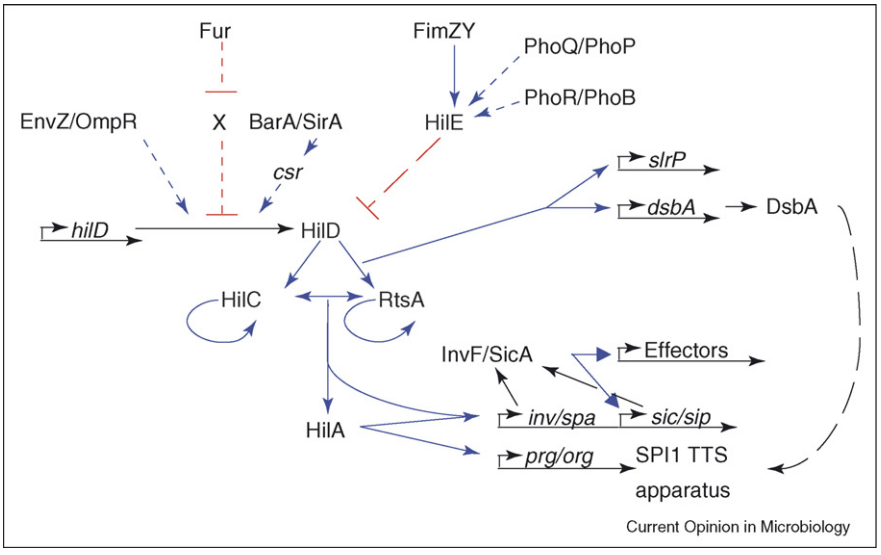
Bacterial pathogens often sense the host environment and respond precisely by expressing gene products required for adaptation to that particular niche. Understanding these adaptive responses and the corresponding regulatory networks is crucial to our overall knowledge of bacterial pathogenesis. *Salmonella* serovars are Gram-negative intracellular pathogens that cause a range of diseases in a variety of hosts. *Salmonella* is typically ingested in contaminated food or water. The bacteria colonize the small intestine and invade normally non-phagocytic epithelial cells in order to gain access to the underlying lymph tissue. Invasion is mediated by a type III secretion system (T3SS) encoded on *Salmonella* pathogenicity island 1 (SPI1). The SPI1 T3SS forms a needle-like complex that is responsible for the injection of bacterial effector proteins into the host cell cytosol. SPI1 effector proteins elicit several physiological changes in the host cell, including actin rearrangement leading to engulfment of the bacterium [1]. The SPI1 T3SS is a prerequisite for symptoms of both abdominal and systemic disease [2–4]. Indeed, the presence of SPI1 is the defining characteristic that distinguishes *Salmonella* from other enterics [5,6]. After invasion of intestinal epithelial cells, *Salmonella* are able to disseminate to any tissue in the body, where the bacteria propagate inside macrophages, a process that requires a second T3SS encoded on *Salmonella* pathogenicity island 2 (SPI2). The SPI2 T3SS effector proteins perform a number functions in the process of maintaining the *Salmonella*-containing vacuole within the macrophage [7,8]. Thus, *Salmonella* encodes two T3SSs on separate pathogenicity islands, and each performs different functions in the overall process of virulence. This complex host–pathogen interaction requires that these systems, along with many additional virulence factors, be precisely controlled, such that each is expressed at the appropriate time and place in the host. The focus of his review is on the regulation of the SPI1 T3SS and its effectors during infection, a model system for understanding adaptation to a particular host environment.

The SPI1 locus is a 40 kb island of *Salmonella*-specific DNA. SPI1 carries all of the genes necessary to create a functional T3SS apparatus, several secreted effectors, and regulators such as HilA, HilC, HilD and InvF (described in more detail below. The structural genes and primary effector proteins are encoded in three operons: *prg/org*, *inv/spa*, and *sic/sip* (Figure 1). The specific roles of the various gene products in the needle complex are being intensely investigated [9–12]. The *prg/org* and *inv/spa* operons encode the needle complex *per se*, whereas the *sic/sip* operon encodes the translocon that embeds in the host cell membrane, as well as the primary effectors. Other injected effectors are encoded elsewhere on the chromosome (reviewed in [13]).

HilA and InvF/SicA

First identified in a mutant that showed a tenfold increase in ability to invade HEp-2 cells, HilA (hyperinvasion locus) is a transcriptional activator that contains a DNA binding motif belonging to the OmpR/ToxR family [14] and a large C-terminal domain of unknown function. HilA is the central regulator in the overall scheme of SPI1 regulation and is known to directly bind to promoters and activate expression of the *prg/org*, and *inv/spa* operons. Read-through from *inv/spa* leads to activation of *sic/sip*

Figure 1



A working model for SPI1 Regulation. Blue arrows indicate activation of gene expression. Repression is noted as red lines with blunt ends. Solid lines represent direct transcriptional regulation. Short-dashed lines represent regulation where the mechanism is not known. Long-dashed lines represent post-translational effects. X represents an unknown intermediate through which Fur controls HilD post-transcriptionally. For clarity, the genes encoding HilC, RtsA, and HilA are not shown. See text for details and references.

[15,16]; thus, HilA activates genes encoding all the components necessary for a functional T3SS [13,14,17,18]. All of the regulatory systems and environmental signals that affect the expression of SPI1 T3SS genes also affect the expression of *hilA* and the data suggest that the level of SPI1 T3SS gene expression is directly dependent on the level of HilA. In BALB/c mouse competition assays, Ellermeier *et al.* [19••] showed that a deletion of *hilA* is phenotypically equivalent to a deletion of the entire SPI1 locus. Another positive transcriptional activator, InvF, is encoded by the first gene in the *inv/spa* operon. InvF is an AraC family member [20] that is capable, with the help of the chaperone SicA, of inducing expression of SPI1 secreted effector proteins encoded both on SPI1 (the *sic/sip* operon) and elsewhere in the chromosome [21,22].

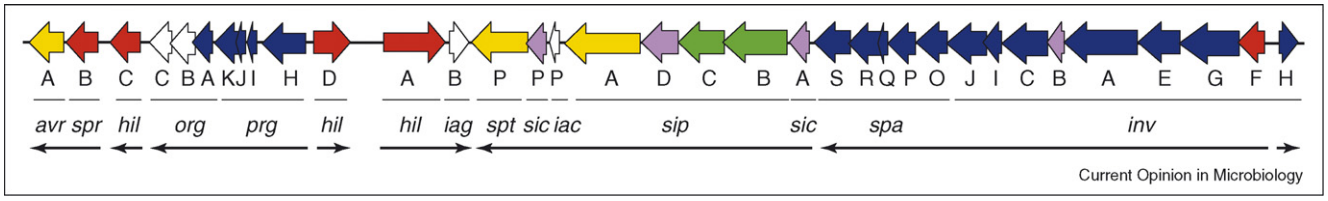
A model for SPI1 regulation

Expression of *hilA* is controlled by the combined action of three AraC-like transcriptional activators: HilC, HilD and RtsA [23–25]. The most significant homology between these proteins is in the C-terminal third, which encodes the DNA binding domain. In this region, RtsA carries 56% identity and 76% similarity to HilC, and 60% identity and 75% similarity to HilD, whereas HilC carries 58% identity and 72% similarity to HilD [25]. The genes encoding HilC and HilD are located on SPI1, whereas RtsA is in encoded on a 15 kb *Salmonella*-specific insert located near the gene for $tRNA^{PheU}$. Studies have shown that HilC, HilD and RtsA can each individually bind to the *hilA* promoter, and deletions of *hilC*, *hilD* or *rtsA* cause a decrease in expression of *hilA* [19••,23–25]. In addition to inducing *hilA* expression, genetic data show that overproduction of HilC, HilD or RtsA can significantly induce expression of *hilC*, *hilD* and *rtsA*. In addition, deletions of *hilC*, *hilD* or *rtsA* cause a decrease in expression of *rtsA* and, to a lesser extent, *hilC* [19••].

These data led us to propose a new model for SPI1 regulation, in which HilC, HilD and RtsA act in a complex feed-forward loop to control expression of *hilA* (Figure 2). As we discuss below, HilD is at the top of the hierarchy. Production of HilD leads to transcriptional activation of HilC and RtsA, which each activate expression of themselves and of each other. All three activators induce expression of *hilA*, and therefore the entire SPI1 T3SS [19••]. HilA negatively autoregulates by an unknown mechanism [26]. Although HilC and RtsA (and HilD) are capable of transcriptionally activating *hilD*, our previous and current data suggest that this is not physiologically relevant and we have removed this facet from our original model. Indeed, our data suggest that production of HilD is largely controlled at the post-transcriptional level, and that this is the key to regulation of SPI1. Various environmental signals, sensed by the regulatory systems, are integrated at the level of HilD production and HilC and RtsA act as amplifiers of the signal. Thus the system acts as a switch, ensuring that SPI1 is fully on at the appropriate time.

HilC, HilD and RtsA, in addition to regulation of *hilA*, are all capable of inducing expression of the *inv/spa* operon — and by read-through, the *sic/sip* operon — in a fashion

Figure 2



SPI1. Needle complex structural genes are indicated in blue; translocon genes are green; effector genes are in yellow; chaperone genes are in purple; regulatory genes are in red; genes of unknown function are white. Gene names are indicated by capital letters (i.e. F represents *invF*) with the locus symbols demarcated by the lines. Arrows below the gene names represent transcripts. The *sitABCD* operon (located downstream of *avrA*) is not included on this slide as it is not involved in the invasion process. See Lostroh and Lee [13], from which the figure was adapted, for references regarding the functional assignment of genes.

independent of HilA [15,16,25,27]. However, HilA is clearly a better inducer of *inv/spa* expression. The genes *slrP* and *dsbA* are also induced by HilC, HilD and RtsA independently of both HilA and InvF [25,28]. SlrP is a T3SS effector of unknown function [29] and DsbA is a periplasmic disulfide bond isomerase that is required for a functional T3SS apparatus [28]. It is certainly possible that other genes are directly regulated by HilC, HilD and RtsA.

Studies have shown that HilC, HilD and RtsA have overlapping binding sites on the *hilA* promoter, but that each has a slightly different DNA recognition sequence [24,30]. This data, along with *in vivo* data from Ellermeier *et al.* [19••] might suggest the presence of mixed HilC, HilD and RtsA dimers. These mixed dimers might account for the fact that HilD alone is not sufficient for activation of *hilA* in competition assays, because a Δ*hilC* Δ*rtsA* strain competes evenly with a Δ*hilD* Δ*hilC* Δ*rtsA* strain [19••]. The mechanism by which HilC, HilD and RtsA activate expression of *hilA* (and possibly of each other) apparently involves counteracting silencing by the nucleoid protein Hns [30,31]. Given the relationship to AraC, it is also possible that these three regulators bind to small molecules that affect regulation. Indeed, the N-terminal regions of the proteins, corresponding to the arabinose-binding domain of AraC, show only 17% identity on average.

Global regulators

SPI1 is a complex regulatory system, and many different signals have been shown to feed into the network. Given the complexity of the system, it is not surprising that data from numerous studies have been previously misinterpreted. Our model provides insight into these previous studies and explains essentially all of the data amassed on this system. Our current understanding suggests that all of the known regulatory systems involved in SPI1 regulation control expression or activity of HilD at some level. We discuss those systems where we have some understanding about how they feed into the SPI1 system (a more substantial list of regulators affecting SPI1 expression is reviewed in [32]).

EnvZ/OmpR

The EnvZ/OmpR two-component regulatory system was classically known to control expression of outer membrane porin genes *ompC* and *ompF* [33]. However, studies have shown that OmpR is a global regulator that also controls many virulence-associated genes [19••,34,35]. Previous studies [35] showed that deletion of *envZ* decreased expression of a *hilC–lacZ* transcriptional fusion, whilst having no effect on expression of a *hilD–lacZ* transcriptional fusion. The interpretation of these data was that OmpR controls *hilC* transcription, a logical conclusion [35]. However, given the feed-forward loop model [19••], it is possible that the OmpR effect on *hilC* transcription was actually mediated through HilD. We examined expression of a *hilA–lac* fusion in Δ*hilC*, Δ*hilD* and/or Δ*rtsA* backgrounds. These results showed that, whereas OmpR-mediated regulation of *hilA* expression was dampened in a Δ*hilC* Δ*rtsA* background, regulation was blocked only in strains missing HilD, suggesting that OmpR controls SPI1 expression by regulating *hilD* expression [19••]. This might not be the result of direct transcriptional activation, but rather of a more complex mechanism, as discussed below.

BarA/SirA

The BarA/SirA two-component regulatory system controls numerous genes involved in carbohydrate metabolism, motility, biofilm formation and invasion [18,35–39]. BarA senses unknown environmental signals — although bile affects regulation by BarA/SirA, and therefore SPI1 expression [40] — and activates the transcriptional regulator SirA. Ellermeier *et al.* [19••] showed that overproduction of SirA can induce expression of a *hilA–lac* transcriptional fusion only when HilD is present. Deletions of *hilC* and *rtsA* alter the total level of *hilA* expression, but do not affect the induction of *hilA* by SirA. This shows a clear requirement for HilD in the SirA induction of SPI1.

Data have shown that SirA acts by inducing expression of two small RNA molecules, *csrB* and *csrC* [36,41,42,43••]. These two small RNAs are antagonistic to the RNA

binding protein CsrA [36,42]. It is probable that CsrA binds to the *hilD* mRNA to either prevent translation or to promote mRNA degradation. Thus SirA induction of *csrBC* prevents CsrA action, indirectly activating *hilD* expression post-transcriptionally [43••]. Indeed, unpublished data from our laboratory show that overproduction of SirA increases expression of HilD only in a *csrA*$^+$ background.

Conversely, previously published gel-shift data [44] suggested that SirA is able to bind to the promoters of *hilC* and *hilA*, but not to that of *hilD*. On the basis of these data, the authors concluded that SirA activates the system by direct action on *hilC* and *hilA* [44]. Whereas SirA might bind to the *hilA* and *hilC* promoters during *in vitro* gel-shift experiments, genetic data shows that SirA is incapable of directly activating these promoters; SirA binding to DNA *in vitro* does not represent activation.

Fur

Fur (ferric uptake regulator), which is the major iron regulator in *Salmonella*, has been recently shown to activate expression of SPI1. Deletion of *fur* causes a decrease in the transcription of *hilA*; overexpression of Fur increases *hilA* transcription dramatically. Mechanistically, Fur is a transcriptional repressor, suggesting that the effects on *hilA* must be indirect. We have shown (JR Ellermeier and JM Slauch, unpublished) that all of the effects are mediated through HilD. Moreover, Fur significantly regulates translational *hilD–lacZ* fusions in a manner that mirrors the effects on *hilA* expression, but it does not affect transcription of *hilD* as monitored by transcriptional *lac* fusions. Thus, although more work is required to understand the mechanism, the regulation of *hilD* is post-transcriptional. Another 'Fur-activated' gene, *sodB*, is controlled at the level of translation by the small RNA *ryhB*, with the help of the RNA binding protein Hfq, in *Escherichia coli* [45–48]. Fur represses *ryhB* transcription [45]. However, deletions of the *ryhB* ortholog (*rfrA*) and paralog (*rfrB*) in *Salmonella enterica* serovar Typhimurium show that *hilD* expression is unaffected by these small RNA molecules, whereas *sodB* is repressed by both *rfrA* and *rfrB* in a seemingly redundant manner (JR Ellermeier and JM Slauch, unpublished). These data suggest that Fur activates SPI1 in a manner different from that of Fur activation of *sodB*. Additionally, Fur is not working through the known SPI1 repressors HilE (see below) or CsrA. As yet, the intermediate through which Fur represses *hilD* translation is unknown. However, given that the effect is apparently at the level of the mRNA, it seems probable that the intermediate is either an RNA binding protein or a small RNA molecule.

HilE

HilE is a major negative regulator of SPI1. Genetic data clearly show that a deletion of *hilE* increases expression of *hilA* only when HilD is present. Although the exact mechanism of HilE action is not clear, data from bacterial two-hybrid studies suggest that HilE binds directly to HilD preventing its action [49]. In *E. coli*, a *hilA–lacZY* fusion was regulated by HilE only when HilD protein was present. Also, when *hilD* is under the control of the *lac* promoter, *hilA* is still regulated by HilE [49]. Whereas the proposed mechanism is based solely on the two-hybrid result, there is no evidence to suggest an alternative model of HilE action.

In turn, *hilE* is regulated by several systems that feed into SPI1. The PhoP/PhoQ and PhoR/PhoB two-component regulatory systems have been shown to have negative regulatory effects on SPI1. FimZ and FimY are regulators that control expression of type 1 fimbriae genes [50]. Recent data from Baxter and Jones [51] have shown that FimZY controls SPI1 expression by regulating *hilE* at the transcriptional level; PhoR/PhoB might regulate SPI1 through *fimZY* [52]. Data suggesting that PhoP represses SPI1 by activating *hilE* expression has also been presented [53]. Thus, the regulatory systems that negatively regulate SPI1 seem primarily to function through HilE.

Regulation of *hilD*

The discovery of post-transcriptional regulation of *hilD* has been a breakthrough. All of the global regulators seem to be controlling *hilA* expression in a HilD-dependent manner. Moreover, it now appears that most, if not all, regulation of SPI1 is mediated post-transcriptionally at *hilD*. Understanding the mechanism(s) of this regulation is the key to the entire system. This regulation is almost certainly indirect. Presumably, the global transcriptional regulators that do not work through HilE control either small RNAs or RNA binding proteins that affect *hilD* translation and/or mRNA stability. With the exception of the SirA-dependent *csr* system, we have not yet identified any of these intermediaries, nor do we know if the remaining systems function through a single intermediary or if each system controls *hilD* independently.

HilC, HilD and RtsA, when overproduced are clearly able to induce *hilD* transcription. However, deletions of *hilC* and/or *rtsA* do not lower the expression level of *hilD* [19••]. Thus, we have removed transcriptional regulation of *hilD* from the model. Interestingly, expression of a translational *hilD–lac* fusion is significantly decreased in a *hilC* and/or *rtsA* mutant. Thus, HilC and RtsA are also apparently indirectly regulating *hilD*, contributing to the feed-forward loop.

Conclusions

The existence of this complex regulatory network emphasizes the precision with which *Salmonella* apparently regulates the SPI1 T3SS. We presume that the various environmental parameters sensed by the global regulatory systems are integrated into SPI1 such that the system is specifically induced when *Salmonella* is in the

small intestine. Under these specific conditions, HilD is produced through the actions of post-transcriptional regulatory mechanisms. This leads to the transcriptional activation of *hilC*, *rtsA* and *hilA*. RtsA and HilC act as amplifiers for the signal, ensuring that the system is turned on rapidly and completely.

Much progress has been made in understanding SPI1 regulation in recent years. However, mysteries and complications abound. We must understand the molecular mechanisms by which *hilD* translation is regulated. Does each of the regulatory systems independently control *hilD* or is there some common intermediary in the process? The feed-forward loop complicates interpretation of experiments and the system must be carefully dissected in order to understand where regulatory signals enter the circuit. The system is further complicated with hints of both positive and negative feedback regulation that are not fully accounted for in the model. With much work ahead, SPI1 will continue to serve as a paradigm for bacterial adaptation to the host environment.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zhou D, Galan J: ***Salmonella* entry into host cells: the work in concert of type III secreted effector proteins**. *Microbes Infect* 2001, **3**:1293-1298.
2. Wallis TS, Galyov EE: **Molecular basis of *Salmonella*-induced enteritis**. *Mol Microbiol* 2000, **36**:997-1005.
3. Tsolis RM, Adams LG, Hantman MJ, Scherer CA, Kimbrough T, Kingsley RA, Ficht TA, Miller SI, Baumler AJ: **SspA is required for lethal *Salmonella enterica* serovar Typhimurium infections in calves but is not essential for diarrhea**. *Infect Immun* 2000, **68**:3158-3163.
4. Zhang S, Santos RL, Tsolis RM, Stender S, Hardt WD, Baumler AJ, Adams LG: **The *Salmonella enterica* serotype Typhimurium effector proteins SipA, SopA, SopB, SopD, and SopE2 act in concert to induce diarrhea in calves**. *Infect Immun* 2002, **70**:3843-3855.
5. Baumler AJ, Tsolis RM, Ficht TA, Adams LG: **Evolution of host adaptation in *Salmonella enterica***. *Infect Immun* 1998, **66**:4579-4587.
6. Porwollik S, Wong RM, McClelland M: **Evolutionary genomics of *Salmonella*: gene acquisitions revealed by microarray analysis**. *Proc Natl Acad Sci USA* 2002, **99**:8956-8961.
7. Gallois A, Klein JR, Allen LA, Jones BD, Nauseef WM: ***Salmonella* pathogenicity island 2-encoded type III secretion system mediates exclusion of NADPH oxidase assembly from the phagosomal membrane**. *J Immunol* 2001, **166**:5741-5748.
8. Vazquez-Torres A, Fang FC: ***Salmonella* evasion of the NADPH phagocyte oxidase**. *Microbes Infect* 2001, **3**:1313-1320.
9. Marlovits TC, Kubori T, Sukhan A, Thomas DR, Galan JE, Unger VM: **Structural insights into the assembly of the type III secretion needle complex**. *Science* 2004, **306**:1040-1042.
10. Marlovits TC, Kubori T, Lara-Tejero M, Thomas D, Unger VM, Galan JE: **Assembly of the inner rod determines needle length in the type III secretion injectisome**. *Nature* 2006, **441**:637-640.
11. Akeda Y, Galan JE: **Chaperone release and unfolding of substrates in type III secretion**. *Nature* 2005, **437**:911-915.
12. Yip CK, Kimbrough TG, Felise HB, Vuckovic M, Thomas NA, Pfuetzner RA, Frey EA, Finlay BB, Miller SI, Strynadka NC: **Structural characterization of the molecular platform for type III secretion system assembly**. *Nature* 2005, **435**:702-707.
13. Lostroh CP, Lee CA: **The *Salmonella* pathogenicity island-1 type III secretion system**. *Microbes Infect* 2001, **3**:1281-1291.
14. Lee CA, Jones BD, Falkow S: **Identification of a *Salmonella typhimurium* invasion locus by selection for hyperinvasive mutants**. *Proc Natl Acad Sci USA* 1992, **89**:1847-1851.
15. Darwin KH, Miller VL: **InvF is required for expression of genes encoding proteins secreted by the SPI1 type III secretion apparatus in *Salmonella typhimurium***. *J Bacteriol* 1999, **181**:4949-4954.
16. Eichelberg K, Galan JE: **Differential regulation of *Salmonella typhimurium* type III secreted proteins by pathogenicity island 1 (SPI-1)-encoded transcriptional activators InvF and HilA**. *Infect Immun* 1999, **67**:4099-4105.
17. Bajaj V, Hwang C, Lee CA: ***hilA* is a novel *ompR/toxR* family member that activates the expression of *Salmonella typhimurium* invasion genes**. *Mol Microbiol* 1995, **18**:715-727.
18. Ahmer BM, van Reeuwijk J, Watson PR, Wallis TS, Heffron F: ***Salmonella* SirA is a global regulator of genes mediating enteropathogenesis**. *Mol Microbiol* 1999, **31**:971-982.
19. •• Ellermeier CD, Ellermeier JR, Slauch JM: **HilD, HilC and RtsA constitute a feed forward loop that controls expression of the SPI1 type three secretion system regulator *hilA* in *Salmonella enterica* serovar Typhimurium**. *Mol Microbiol* 2005, **57**:691-705.
This work demonstrates the regulation of *rtsA*, *hilC*, *hilD*, and *hilA* by HilC, HilD and RtsA, and that each regulator has a role in the host during infection. It also demonstrates BarA/SirA and EnvZ/OmpR control of *hilA* through HilD. These data form the basis of our current model of SPI1 regulation.
20. Martin RG, Rosner JL: **The AraC transcriptional activators**. *Curr Opin Microbiol* 2001, **4**:132-137.
21. Darwin KH, Miller VL: **The putative invasion protein chaperone SicA acts together with InvF to activate the expression of *Salmonella typhimurium* virulence genes**. *Mol Microbiol* 2000, **35**:949-960.
22. Darwin KH, Miller VL: **Type III secretion chaperone-dependent regulation: activation of virulence genes by SicA and InvF in *Salmonella typhimurium***. *EMBO J* 2001, **20**:1850-1862.
23. Schechter LM, Lee CA: **AraC/XylS family members, HilC and HilD, directly bind and derepress the *Salmonella typhimurium hilA* promoter**. *Mol Microbiol* 2001, **40**:1289-1299.
24. Olekhnovich IN, Kadner RJ: **DNA-binding activities of the HilC and HilD virulence regulatory proteins of *Salmonella enterica* serovar Typhimurium**. *J Bacteriol* 2002, **184**:4148-4160.
25. Ellermeier CD, Slauch JM: **RtsA and RtsB coordinately regulate expression of the invasion and flagellar genes in *Salmonella enterica* serovar Typhimurium**. *J Bacteriol* 2003, **185**:5096-5108.
26. De Keersmaecker SC, Marchal K, Verhoeven TL, Engelen K, Vanderleyden J, Detweiler CS: **Microarray analysis and motif detection reveal new targets of the *Salmonella enterica* serovar Typhimurium HilA regulatory protein, including *hilA* itself**. *J Bacteriol* 2005, **187**:4381-4391.
27. Lostroh CP, Lee CA: **The HilA box and sequences outside it determine the magnitude of HilA-dependent activation of P(*prgH*) from *Salmonella* pathogenicity island 1**. *J Bacteriol* 2001, **183**:4876-4885.
28. Ellermeier CD, Slauch JM: **RtsA coordinately regulates DsbA and the *Salmonella* pathogenicity island 1 type III secretion system**. *J Bacteriol* 2004, **186**:68-79.
29. Tsolis RM, Townsend SM, Miao EA, Miller SI, Ficht TA, Adams LG, Baumler AJ: **Identification of a putative *Salmonella enterica***

serotype Typhimurium host range factor with homology to IpaH and YopM by signature-tagged mutagenesis. *Infect Immun* 1999, **67**:6385-6393.

30. Olekhnovich IN, Kadner RJ: **Crucial roles of both flanking sequences in silencing of the *hilA* promoter in *Salmonella enterica***. *J Mol Biol* 2006, **357**:373-386.

31. Schechter LM, Jain S, Akbar S, Lee CA: **The small nucleoid-binding proteins H-NS, HU, and Fis affect *hilA* expression in *Salmonella enterica* serovar Typhimurium**. *Infect Immun* 2003, **71**:5432-5435.

32. AltierC: **Genetic and environmental control of *Salmonella* invasion**. *J Microbiol* 2005, 43 Spec No:85-92.

33. Pratt LA, Hsing W, Gibson KE, Silhavy TJ: **From acids to *osmZ*: multiple factors influence synthesis of the OmpF and OmpC porins in *Escherichia coli***. *Mol Microbiol* 1996, **20**:911-917.

34. Feng X, Walthers D, Oropeza R, Kenney LJ: **The response regulator SsrB activates transcription and binds to a region overlapping OmpR binding sites at *Salmonella* pathogenicity island 2**. *Mol Microbiol* 2004, **54**:823-835.

35. Lucas RL, Lee CA: **Roles of *hilC* and *hilD* in regulation of *hilA* expression in *Salmonella enterica* serovar Typhimurium**. *J Bacteriol* 2001, **183**:2733-2745.

36. Romeo T: **Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB**. *Mol Microbiol* 1998, **29**:1321-1330.

37. Johnston C, Pegues DA, Hueck CJ, Lee A, Miller SI: **Transcriptional activation of *Salmonella typhimurium* invasion genes by a member of the phosphorylated response-regulator superfamily**. *Mol Microbiol* 1996, **22**:715-727.

38. Rakeman JL, Bonifield HR, Miller SI: **A HilA-independent pathway to *Salmonella typhimurium* invasion gene transcription**. *J Bacteriol* 1999, **181**:3096-3104.

39. Lawhon SD, Frye JG, Suyemoto M, Porwollik S, McClelland M, Altier C: **Global regulation by CsrA in *Salmonella typhimurium***. *Mol Microbiol* 2003, **48**:1633-1645.

40. Prouty AM, Gunn JS: ***Salmonella enterica* serovar Typhimurium invasion is repressed in the presence of bile**. *Infect Immun* 2000, **68**:6763-6769.

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

42. Weilbacher T, Suzuki K, Dubey AK, Wang X, Gudapaty S, Morozov I, Baker CS, Georgellis D, Babitzke P, Romeo T: **A novel sRNA component of the carbon storage regulatory system of *Escherichia coli***. *Mol Microbiol* 2003, **48**:657-670.

43. •• Fortune DR, Suyemoto M, Altier C: **Identification of CsrC and characterization of its role in epithelial cell invasion in *Salmonella enterica* serovar Typhimurium**. *Infect Immun* 2006, **74**:331-339.

This work demonstrates the effects of the CsrA-antagonistic small RNAs CsrB and CsrC, which alter the levels of SPI1 gene expression and *Salmonella* invasion. In addition, the authors demonstrate that deletions of both *csrB* and *csrC* combine to give an invasion defect similar to that of a *sirA* deletion, suggesting SirA-mediated SPI1 regulation occurs entirely through the *csr* system.

44. Teplitski M, Goodier RI, Ahmer BM: **Pathways leading from BarA/SirA to motility and virulence gene expression in *Salmonella***. *J Bacteriol* 2003, **185**:7257-7265.

45. Masse E, Gottesman S: **A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli***. *Proc Natl Acad Sci USA* 2002, **99**:4620-4625.

46. Vecerek B, Moll I, Afonyushkin T, Kaberdin V, Blasi U: **Interaction of the RNA chaperone Hfq with mRNAs: direct and indirect roles of Hfq in iron metabolism of *Escherichia coli***. *Mol Microbiol* 2003, **50**:897-909.

47. Geissmann TA, Touati D: **Hfq, a new chaperoning role: binding to messenger RNA determines access for small RNA regulator**. *EMBO J* 2004, **23**:396-405.

48. Masse E, Vanderpool CK, Gottesman S: **Effect of RyhB small RNA on global iron use in *Escherichia coli***. *J Bacteriol* 2005, **187**:6962-6971.

49. Baxter MA, Fahlen TF, Wilson RL, Jones BD: **HilE interacts with HilD and negatively regulates *hilA* transcription and expression of the *Salmonella enterica* serovar Typhimurium invasive phenotype**. *Infect Immun* 2003, **71**:1295-1305.

50. Yeh KS, Tinker JK, Clegg S: **FimZ binds the *Salmonella typhimurium fimA* promoter region and may regulate its own expression with FimY**. *Microbiol Immunol* 2002, **46**:1-10.

51. Baxter MA, Jones BD: **The *fimYZ* genes regulate *Salmonella enterica* serovar Typhimurium invasion in addition to type 1 fimbrial expression and bacterial motility**. *Infect Immun* 2005, **73**:1377-1385.

52. Jones BD: ***Salmonella* invasion gene regulation: a story of environmental awareness**. *J Microbiol* 2005, 43 Spec No:110-117.

53. Baxter M, Jones BD: **Identification of regulatory pathways that translate environmental signals into changes in expression of *Salmonella* motility, adherence, and invasion [abstract]**. *103rd General Meeting of the American Society for Microbiology* 2003, abstr. D-110.