

# Virulence gene regulation in *Salmonella enterica*

Mark Clements<sup>1</sup>, Sofia Eriksson<sup>1</sup>, Dilek Tezcan-Merdol<sup>1</sup>, Jay C D Hinton<sup>2</sup>  
and Mikael Rhen<sup>1</sup>

In order to infect a host, a microbe must be equipped with special properties known as virulence factors. Bacterial virulence factors are required to facilitate colonization, to survive under host defenses, and to permit multiplication inside the host. However, the possession of genes encoding virulence factors does not guarantee effective infection. There is considerable evidence that tight regulation of a given virulence factor is as important as the possession of the virulence factors themselves. Thus, an understanding of the regulation of virulence expression is fundamental to our comprehension of any infection process and can identify potential targets for disease prevention and therapy. We have summarized the lessons learned from experimental salmonellosis in terms of virulence regulation and hope to illustrate the differing requirements for gene and virulence expression.

Keywords: gene regulation; pathogenesis; *Salmonella*; sensor; virulence.

*Ann Med* 2001; 33: 178–185.

## ***Salmonella enterica*: a pathogen and a laboratory tool**

*S. enterica* can be defined as a collection of closely related enteric bacteria which, 'as a species', is capable of infecting a wide variety of hosts, including man. Because they are typical 'enteric bacteria', salmonellae have been used for decades as laboratory model organisms in the fields of biochemistry, genetics, pathogenesis, and vaccine development (1).

The annual incidence of the most severe form of human salmonellosis, typhoid fever, approaches 16

million cases, whereas the rate of salmonella-related gastroenteritis is much higher (2). *Salmonella* infection follows ingestion of contaminated food, water or beverages and requires survival in the stomach and colonization of the small intestine. At this stage, the bacteria are seen to start multiplying and adhering to the intestinal mucosa. In the mouse model system, invasion of the intestinal epithelial cell barrier by *S. enterica* serovar (sv) Typhimurium is an essential step for systemic salmonellosis (3). Once the epithelial cell barrier has been penetrated, the bacteria spread through the mesenteric lymphatic system and the blood stream to the liver and spleen. It is noteworthy that this scenario described for mice resembles the course of typhoid fever caused by *S. enterica* sv Typhi in man (4).

The intimate interaction between bacteria and host cells is the central theme of a *Salmonella* infection. It not only involves adhesion to host epithelial cells (5, 6), but also the induction of cytokine and inflammatory responses (7, 8) and active invasion of host cells (9, 10). Careful microscopic examination of infected livers of mice (11) and rats (12) indicates that during the later stages of a systemic infection the bacteria reside preferentially within the Kupffer cells. There is strong evidence that subsequent bacterial intracellular multiplication is a prerequisite for bacterial lethality and virulence (11–15). This explains why *Salmonella* is such a valuable research tool, not only as a model for studying human typhoid fever but also for developing our understanding of bacterial intracellular parasitism in general. Accordingly, a number of virulence factors have been identified and characterized in sv Typhimurium, that contribute to bacterial virulence in mice. These include fimbrial adhesive organelles, which enable the bacteria to attach to host cell surfaces, and secreted effector proteins, which aid bacterial invasion and promote intracellular bacterial survival. Many of the gene functions responsible for bacterial infection are not expressed under ordinary laboratory conditions but are induced for expression under special culture conditions or in milieus resembling the host environment (1, 5, 6, 9, 10).

From the <sup>1</sup>Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, the Swedish Institute for Infectious Disease Control, Solna, Sweden; and the <sup>2</sup>Institute of Food Research, Norwich Research Park, Norwich, UK.

Correspondence: Mikael Rhen, PhD, Microbiology and Tumor Biology Center, Karolinska Institute, Nobels väg 16, 171 77 Stockholm, Sweden. E-mail: mikael.rhen@mtc.ki.se; Fax: +46 8 301797.

This regulation of expression of virulence factors is the key to success of *Salmonella* as a pathogen and the subject of concerted research effort worldwide. Perhaps the regulation of virulence expression should be considered a virulence factor in itself.

## ***Salmonella*, gene regulation and virulence**

### *Gene regulators that react to environmental changes*

A number of central gene regulatory functions govern the life and growth of salmonellae, and these have been studied for decades. In fact, many important regulatory circuits were initially described in this bacterium. Some regulatory circuits are involved in the tuning of ordinary house-keeping functions and are likewise present in *Escherichia coli*, whereas many appear to be devoted to virulence or function as global gene regulators affecting both house-keeping and virulence functions (Table 1).

Although salmonellae lack the classic *lac* regulatory system, they possess the well-characterized catabolite repressor system (1). Catabolite repression responds to a small-molecular-weight signal in the form of cyclic adenosine monophosphate (cAMP). Bacterial intracellular concentration of cAMP increases as a result of the shortage of certain nutrients, a situation

### **Key messages**

- Adaptation of *Salmonella enterica* to host milieu involves sensing of environmental changes and subsequent co-ordinated expression of virulence genes.
- A meaningful induction of virulence genes involves the concerted action of global gene regulators and specific virulence gene regulators.
- Functional inactivation of virulence gene regulation may lead to avirulence.

that can resemble starvation. The gene regulatory catabolite repressor protein (CRP) responds to changes in intracellular cAMP concentrations by binding the ligand cAMP. Binding of CRP to cAMP modulates its DNA-binding ability; depending on the promoter in question a cAMP–CRP complex can act either as an activator or repressor to control gene expression with the aim of counteracting starvation. Four details of the CRP response are relevant to the regulation of bacterial virulence gene regulation. First, a gene regulatory protein is involved, which is allosteric in its function. Second, a signal substance modulates the

Table 1. Examples of regulators of *Salmonella enterica* virulence expression.

Regulator	Environment/signal sensed	Targets	Effect(s) on virulence
CRP	Starvation, cAMP	Multiple, <i>spv</i> (via <i>rpoS</i> ?)	Many
LysR/MetR-type regulators			
SprR	Starvation, intracellular compartment	<i>spvRABCD</i>	Increased intracellular replication
AraC-type regulators			
SprA	Intestine?	SPI1 genes	Increased invasion
InvF	Intestine?	SPI1 genes	Invasion
Two-component regulatory systems			
HilA	Intestine?	SPI1 genes	Invasion
OmpR/EnvZ	Osmolarity, pH	Multiple	Many, involved in the induction of stress tolerance
PhoP/PhoQ	Mg <sup>2+</sup> /Ca <sup>2+</sup> , pH	Multiple, (eg <i>pag</i> , <i>prg</i> , <i>spv</i> , SPI2 genes)	Many, involved in the induction of stress tolerance, suppression of invasion, induction of intracellular survival and growth.
RcsB/RcsC	Osmolarity	Multiple, (eg <i>invF</i> , <i>sjpB</i> )	Alteration in SPI-1, flagellin and Vi-antigen expression
Alternative $\sigma$ factors			
RpoS	Starvation	Multiple, (eg <i>spv</i> )	Many, induction of stress tolerance, increased intracellular survival and growth
Others			
H-NS	Temperature, (DNA topology)	Global gene (co)regulator	Many
TlpA	Temperature	<i>tlpA</i>	Unknown
Fur	Iron, pH	Multiple, (eg <i>sitABCD</i> )	Many, involved in the induction of stress tolerance

activity of the gene regulatory protein. Third, the CRP response is essential for the virulence of salmonella (16). Finally, catabolite repression is an example of the ability of the bacterium to sense environmental changes or stresses and to give rise to a specific response.

In addition to CRP–cAMP, there are many protein families defined in *S. enterica* sv Typhimurium that have the ability to sense environmental changes. These include the so-called LysR/MetR regulatory protein family, the AraC protein family, the iron response-regulator Fur family, and two-component regulatory systems.

*LysR/MetR gene regulators.* These regulatory proteins are characterized by an N-terminal DNA-binding helix-turn-helix motif, followed by a central protein domain that binds small-molecular-weight coregulatory molecules (17) or is capable of responding to oxidative substances (18). Some members of the LysR/MetR family also contain a cationic C-terminus that may be involved in DNA binding. Like the CRP–cAMP response, the activity of these regulatory proteins is affected by the presence of a coregulatory molecule, and many LysR/MetR members are involved in the feed-back regulation of metabolic pathways.

Based on protein sequence identity, the SpvR protein associated with intracellular growth of *Salmonella* clearly belongs to the LysR/MetR family (19–21).

*AraC-type gene regulators.* This large family of gene regulators contains characteristic C-terminal helix-turn-helix motifs involved in DNA binding (22). The N-terminal portions are variable, presumably reflecting different evolutionary backgrounds and functional attributes. The prototype regulator AraC is involved in the regulation of arabinose catabolism; the sugar arabinose binds to AraC and modulates its activity (1). Thus, this family of regulators also has the potential to sense environmental factors. Not surprisingly, therefore, many members of the AraC family are known to be involved in virulence gene expression.

In salmonellae, some virulence functions are encoded by large clusters of genes. One such constellation of genes constitutes the pathogenicity island 1 (SPI 1), which is required for the invasion of epithelial cells. The expression of a number of genes on SPI 1 is regulated by the AraC-like proteins InvF and SprA (23–26).

*Two-component regulatory systems.* For this group of regulators, the necessary sensor and regulatory domains are usually contained in separate proteins (1, 27). The sensor domain is normally located in the periplasmic membrane and, as a result of environ-

mental changes, phosphorylates the cytoplasmic regulatory component. The promoter specificity and regulatory capacity of the regulator component become altered through the phosphorylation. This enables the bacterium to sense changes occurring outside the cytosolic compartment and to subsequently transmit information into the cytosol in order to achieve a gene response. Changes in osmolarity and Ca<sup>++</sup>/Mg<sup>++</sup> concentration are sensed by the OmpR/EnvZ and PhoP/PhoQ sensor regulator pairs, respectively, both essential for *Salmonella* virulence (28–30). The SPI 1 pathogenicity gene island encodes an activator protein HilA that belongs to this family of regulators and is important for the expression of bacterial invasion functions, whereas invasion and Vi surface antigen expression in sv Typhi is controlled by the regulator homologue RcsB (31).

Several important virulence-associated gene regulators of *Salmonella* belong to the so-called LuxR family (32). Some members of this family function as the regulator component for other sensor/kinase components. Otherwise, the LuxR family of regulators responds to autoinducer molecules, which are involved in the process of quorum sensing. Interestingly, SPI 1 codes for a protein SprB, which shares homology with the LuxR regulator proteins (33).

*Sensing changes in temperature.* While the above gene regulators illustrate how bacteria can sense changes in chemical composition, other examples are required to demonstrate how bacteria sense changes in temperature. While temperature-dependent gene control is complex and multifactorial, involving changes in DNA topology and RNA configuration and RNA half-life, it does involve several regulatory proteins (34).

An apparently *Salmonella*-specific temperature-sensing gene regulator is the TlpA protein (35). TlpA has been shown to bind a specific region in the promoter region of *tlpA*. Closely related sequence motifs are found in the promoter regions of four virulence genes (*orgA*, *pagC*, *prgH* and *spvA*) and two genes (*bipA* and *envM*) responding to exposure to bactericidal components. What makes TlpA particularly interesting is that the temperature-sensing and DNA-binding regions reside in the same molecule. The TlpA protein consists of two main domains: an N-terminal DNA-binding domain is followed by an extended region allowing the protein to form coiled-coil dimers. Dimer formation is subjected to a temperature- and monomer concentration-dependent equilibrium, and only the dimer binds DNA to cause repression of gene expression. An elevation in temperature, eg from 28 °C to 43 °C, will result in dissociation of dimers into monomers and consequent transcriptional activation of the *tlpA* promoter.

### *Global gene regulation, host adaptation and induction of virulence gene expression*

**Alterations in DNA topology.** The topological structure of the bacterial chromosome is not linear, but involves a compact supercoiled structure (36, 37). The degree of global DNA supercoiling is not constant, but varies as a response to changes in environmental conditions (38, 39). The activity of many promoters is sensitive to changes in DNA topology, and these control many environmentally regulated genes that are commonly involved in virulence (40, 41). Thus, the activities of a number of genes and subsequently the physiology of the cell can be altered through changes in DNA supercoiling. Two enzymes are mainly responsible for altering DNA supercoiling; DNA gyrase (topoisomerase II) and topoisomerase I (1, 41). The former introduces negative supercoils into DNA, whereas the latter catalyses relaxation or unwinding of supercoiled DNA. As the activity of DNA gyrase is dependent on the cellular ATP/ADP ratio, changes in metabolic status are translated into changes in the level of DNA supercoiling (42). Also, the promoters directing gyrase and topoisomerase expression respond directly to changes in DNA supercoiling.

In addition to the above two enzymes there are DNA-binding proteins that constrain or maintain DNA supercoiling, such as H-NS. H-NS is a small protein that binds DNA in a nonsequence-specific manner (43, 44). H-NS generally causes repression of gene expression and is known to affect the activity of a number of virulence-associated promoters (44–47). H-NS resembles TlpA in its ability to repress promoter activity at low temperature (34). Crucially, H-NS is an oligomeric protein (44), which forms hetero-oligomers with the other H-NS-like protein StpA in *E. coli* and *S. enterica* sv Typhimurium (44). It is possible that H-NS activity can be modulated through alterations in oligomeric state or through its binding to DNA.

Another important protein that binds throughout the genome is the integration host factor (IHF). In contrast to H-NS, IHF shows clear sequence specificity for binding (43). IHF helps to create DNA loops and to form protein–DNA complexes at promoter sites (48, 49), which have specific effects on local transcription.

**Altering the composition of the RNA polymerase complex.** In bacteria, alternative  $\sigma$  factors are expressed under certain stressful environmental conditions. In *Salmonella* (50), as well as in many other bacteria, the alternative  $\sigma$  factor RpoS is expressed as a response to nutrient starvation or a sudden decrease in pH (51). RpoS can replace the vegetative  $\sigma$  factor leading to an altered RNA polymerase complex that recognizes a specific subset of promoters. Con-

sequently, the expression of a number of genes is affected (52, 53). In *Salmonella*, starvation and subsequent expression of RpoS is a prerequisite for the expression of many virulence factors, such as AgfA adhesive fibres (54) and the Spv intracellular growth proteins (50, 55). Also, at least in *E. coli*, the expression of IHF is dependent on RpoS (56).

**DNA methylation.** The DNA in wild-type strains of *Salmonella* is methylated to varying degrees. Inactivation of the Dam methylation system in *S. enterica* sv Typhimurium, which as in *E. coli*, acts on GATC sequences, drastically reduces virulence in mice (57). In addition, Dam mutants are impaired in their ability to invade host cells and in their levels of expression of Pef fimbriae (47). Genetic analyses by using *pef* promoter sequences show that the promoter DNA must be methylated at specific GATC sequences for proper transcription to occur. As the expression of invasion and Pef are strongly dependent on environmental cues, alterations in the degree of methylation at promoter sequences may well be a tool for tuning gene expression.

### **Acid induction of gene expression**

Exposing *Salmonella* to sudden temperature increase, reactive oxygen compounds, low pH or nutrient starvation induces defined stress responses that cause specific effects on global bacterial gene expression. Evidently, the purpose of these responses is to allow bacteria to adapt to new extreme environments or to specific shortages in nutrients. For example, the sudden exposure of a *Salmonella* culture growing at neutral pH (~ pH 7) to low pH (~ pH 3) is detrimental for the culture. However, if the culture growing at neutral pH is pre-exposed to a more moderate pH shift (~ pH 5), it will be able to cope with subsequent sudden drops in pH (58). It is clear that the result of a moderate pH stress is the induction of expression of certain bacterial functions required for adaptation to an acidic environment (58, 59), a process called the acid tolerance response (ATR). The induction of ATR improves the ability of bacteria to handle an acid gradient and is evidently required for the intracellular multiplication of *S. enterica* sv Typhimurium in the acidified phagosomal vacuole of macrophages.

Given that the intracellular environment can be harsh for bacteria, it is not surprising that there is an overlap between the induction of general stress responses and virulence (60–62). For example, heat shock response can be induced in *E. coli* by increasing the growth temperature from 30 °C to 42 °C, with a concomitant induction of some 20 proteins. In parallel, many heat shock-induced proteins are also switched

on during growth of *Salmonella* within eukaryotic cells (34, 63) implying that (parts of) this stress response can also be induced by host environment. Interestingly, many crucial components of the starvation response and the ATR are also well-defined virulence regulators (64, 65) and involve, as regulators, components such as OmpR, RpoS, PhoP/PhoQ and Fur, which are also found in *E. coli*. Furthermore, the ATR is a useful example, as it has pleiotropic effects, allowing the bacteria to adapt to various types of acids, to the host environment and, by involving the combined action of several gene regulators, to affect the expression of more than 50 genes (66). One can distinguish two types of ATRs. One is induced in the logarithmic growth phase, the other occurs as the culture enters the stationary phase. The log-phase ATR includes OmpR, the alternative  $\sigma$  factor RpoS, and the iron response regulator Fur for induction (64, 67, 68). The stationary-phase ATR includes at least 10 genes separate from log-phase ATR (69) and is dependent on the regulator OmpR (70).

The log-phase ATR involves separate adaptations for inorganic and organic acids, in which PhoP appears to have a central role in establishing tolerance to inorganic acid stress (71). What is interesting in this context is that three components defined as regulatory factors in the ATRs, OmpR, Fur and PhoP, were initially identified as regulators responding to osmolarity or to selected metal ions. Thus, either pH changes affect the ability of corresponding regulatory circuits to sense the environmental ion content, or alternatively the circuits have dual sensing ability.

### Regulating an infection: fine-tuning the expression of virulence

It seems logical that bacteria would induce virulence factors when times are hard, and that environmental stress would have a role as an inducer. However, stressful conditions are certainly experienced outside hosts, and a simple on-off induction of general stress responses, including concomitant induction of virulence factors, would appear both misdirected and futile. In fact, many stress responses are multilayered; the final outcome reflects the co-ordinate interplay between many regulatory circuits.

As the salmonellae enter the host through contaminated food, the acidic content of the stomach represents the first major host innate defense. Previous starvation may well have induced the expression of RpoS and RpoS-mediated responses in the infecting bacteria, allowing a proportion to survive the acid shock (61). For many *Salmonella* strains, low growth temperature and RpoS induce the expression of thin aggregative fibres (54), or AgfA fimbriae, which the

bacteria may use for adhering to intestinal epithelial cells (72, 73). Thus, environmental starvation could serve as a priming factor for bacterial adaptation to the host.

To establish a systemic infection, the surviving and adhering bacteria in the small intestine must initiate invasion of the epithelial cell barrier. Invasion of epithelial cells is not a passive process, and salmonellae are equipped with special functions enabling them to inject effector proteins into the host cell cytoplasm (74, 75). One function of these SPI 1-encoded proteins is to hijack the nonphagocytic cells to force the endocytosis of bacteria from the lumen. In addition to participating in the generation of the ATR, the *phoP/phoQ* regulon also controls invasion of epithelial cells and the survival of bacteria in phagocytic cells (29, 30, 76). As the concentration of  $Mg^{++}$  decreases, possibly as a result of entering a host cell, the *phoP/phoQ* regulon activates the expression of a set of genes, collectively termed *pags*, that adapt the bacterium to intracellular life (77). At the same time, another set of genes, the *prgs*, that are needed for bacterial invasion become repressed (76, 77).

The importance of this regulation cascade is illustrated by the significant attenuation caused by genetical inactivation of the PhoP/PhoQ regulation system (29, 30). The fact that the constant activation of PhoP by a constitutive PhoQ mutant also causes a decrease in virulence (78) further illustrates the importance of the PhoP/PhoQ regulation of virulence gene expression during an infection. We need to understand how the *phoP/phoQ* regulon distinguishes between induction of the ATR and of effector proteins required for invasion. One obvious solution would be to include down-stream regulatory functions that control invasion gene regulation and respond to PhoPQ. In fact, the SPI 1 invasion gene cluster has several regulatory functions, including the HilA activator protein that switches on the expression of invasion genes (79, 80). The expression of *hilA* is itself dependent on genes within and outside the invasion gene cluster (79, 80). For example, the response regulator SirA has been shown to activate *hilA* transcription in the presence of suitable environmental signals (79, 80), providing us with tools for both an on- (SirA/HilA) and off-switch (PhoP-mediated *prg* response) for invasion.

In experimental systems, the *spv* intracellular growth genes are dependent on RpoS for expression (20, 21). However, the production of RpoS alone is not sufficient for *spv* expression. This makes sense because intracellular location is defined by more than starvation. Full induction of *spv* expression has been shown to depend on RpoS, SpvR, H-NS, IHF and a global regulatory component termed leucine response protein (LRP) (81, 82). Although the function of each component in this complex concerted regulatory

process is not absolutely clear, their purpose is evidently to ensure that *spv* genes are expressed only at the site of action, which is when the bacteria have entered a host cell and should start their intracellular multiplication phase. In *S. enterica* sv Typhimurium, *spv* genes are strongly induced for transcription in cultured host cells, while this induction is abolished in *spvR* mutants. This has led to the suggestion that induction of *spv* virulence genes would be initiated by the binding of SpvR to a (as yet unidentified) ligand specifying the intracellular milieu, and that this SpvR–ligand complex would act as a key activator of Spv protein expression (20, 21).

As well as being regulated at the transcriptional level, bacterial gene products are subjected to post-transcriptional regulation both at the mRNA and the protein level. Some observations suggest that post-translational gene regulation would play a role in *Salmonella* virulence. The RpoS protein of *S. enterica* sv Typhimurium, for example, is subject to post-transcriptional regulation (83). Also, deletion of the *E. coli* homologue *csrB*, involved in RNA turnover, from *S. enterica* sv Typhimurium has an effect on invasion gene expression (84).

## Conclusion

These examples demonstrate that bacteria are sensitive to both temporal and environmental factors during an infection by use of combinations of regulatory systems with built-in sensory capabilities (Table 1). Exactly which environmental cues these systems sense and the definition of the complex patterns of regulatory interplay remain to be elucidated. At the level of the promoter itself, many regulatory components make up defined nucleo–protein complexes that accumulate in the presence of certain environmental stimuli. In such complexes, the balance and the functional role of individual regulatory proteins may be modified to achieve the final level of gene control that is required for successful infection.

It is becoming clear that to understand the biology of bacterial infection we must stop relying upon the reductionist process of determining the role of individual virulence gene regulators and instead look towards a more integrated approach. The recent completion of the *S. enterica* sv Typhimurium genome sequence may provide the additional methodology (85) and the key information we have been looking for.

## References

1. Neidhardt FC, (ed). *Escherichia coli* and *Salmonella*: cellular and molecular biology. Washington, DC: ASM Press; 1999.
2. Pang T, Bhutta ZA, Finlay BB, Altwegg M. Typhoid fever and other salmonellosis: a continuing challenge. *Trends Microbiol* 1995; 3: 253–5.
3. Carter PB, Collins FM. The route of enteric infection in normal mice. *J Exp Med* 1974; 139: 11889–203.
4. Owen RL. M-cells – enterways of opportunity for enteropathogens. *J Exp Med* 1994; 180: 7–9.
5. Sukupolvi S, Lorenz RG, Gordon JI, Bian Z, Pfeifer JD, Normark SJ, et al. Expression of thin aggregative fimbriae promotes interaction of *Salmonella typhimurium* SR-11 with mouse intestinal epithelial cells. *Infect Immun* 1997; 65: 5320–5.
6. Van der Velden AW, Bäumlér AJ, Tsolis RM, Heffron F. Multiple fimbrial adhesins are required for full virulence of *Salmonella typhimurium* in mice. *Infect Immun* 1998; 66: 2803–8.
7. Weinstein DL, O'Neill BL, Hone DM, Metcalf ES. Differential early interactions between *Salmonella enterica* serovar Typhi and two other pathogenic *Salmonella* serovars with intestinal epithelial cells. *Infect Immun* 1998; 66: 2310–8.
8. Hobbie S, Chen LM, Davis RJ, Galán JE. Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by *Salmonella typhimurium* in cultured intestinal epithelial cells. *J Immunol* 1997; 159: 5550–9.
9. Ginocchio CC, Olmsted SB, Wells CL, Galán JE. Contact with epithelial cells induces the formation of surface appendages of *Salmonella typhimurium*. *Cell* 1994; 76: 717–24.
10. Galán JE. Molecular genetic bases of *Salmonella* entry into host cells. *Mol Microbiol* 20: 263–71.
11. Richter-Dahlfors A, Buchan AMJ, Finlay BB. Murine salmonellosis studied by confocal microscopy: *Salmonella typhimurium* resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes *in vivo*. *J Exp Med* 1997; 186: 569–80.
12. Nahlue NA, Shnyra A, Hultenby K, Lindberg AA. *Salmonella choleraesuis* and *Salmonella typhimurium* associated with liver cells after intravenous inoculation of rats are localized mainly in the Kupffer cells and multiply intracellularly. *Infect Immun* 1992; 60: 2758–68.
13. Fields PI, Swanson RV, Haidaris CG, Heffron F. Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. *Proc Natl Acad Sci U S A* 1986; 83: 5189–93.
14. Lissner CR, Swanson RN, O'Brien A. Genetic control of the innate resistance of mice to *Salmonella typhimurium*: expression of the *Ity* gene in peritoneal and splenic macrophages isolated *in vitro*. *J Immunol* 1983; 131: 3006–13.
15. Gulig PA, Doyle TJ, Hughes JA, Matsui H. Analysis of host cells associated with the Spv-mediated increased growth rate of *Salmonella typhimurium* in mice. *Infect Immun* 1998; 2471–85.
16. Curtiss R III, Kelly SM. *Salmonella typhimurium* deletion mutants lacking adenylate cyclase and cyclic AMP receptor protein are avirulent and immunogenic. *Infect Immun* 1987; 55: 3035–43.
17. Schell M A. Molecular biology of the LysR family of transcriptional regulators. *Ann Rev Microbiol* 1993; 47: 597–626.
18. Aslund F, Zheng M, Beckwith J, Sorz G. Regulation of the OxyR transcription factor by hydrogen peroxide and the

- cellular thiol-disulfide status. *Proc Natl Acad Sci U S A* 1999; 96: 6161–5.
19. Gulig PA, Danbara H, Guiney DG, Lax AJ, Norel F, Rhen M. Molecular analysis of the *spv* virulence genes of the salmonella virulence plasmids. *Mol Microbiol* 1993; 7: 825–30.
  20. Guiney DG, Libby S, Fang F, Krause M, Fierer J. Growth-phase regulation of plasmid virulence genes in *Salmonella*. *Trends Microbiol* 1995; 3: 275–9.
  21. Heiskanen P, Taira S, Rhen M. Role of *rpoS* in the regulation of *Salmonella* plasmid virulence (*spv*) genes. *FEMS Microbiol Lett* 1994; 123: 125–30.
  22. Gallegos MT, Schleif R, Bairoch A, Hofmann K, Ramos JL. AraC/XylS family of transcriptional regulators. *Microbiol Mol Biol Rev* 1997; 61: 393–410.
  23. Eichelberg K, Hardt WD, Galán JE. Characterization of SprA, an AraC-like transcriptional regulator encoded within the *Salmonella typhimurium* pathogenicity island 1. *Mol Microbiol* 1999; 33: 139–52.
  24. 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: 4949–54.
  25. Schechter LM, Damrauer SM, Lee CA. Two AraC/XylS family members can independently counteract the effect of repressing upstream of the *hilA* promoter. *Mol Microbiol* 1999; 32: 629–42.
  26. Rakeman JL, Bonifield HR, Miller SI. A HilA-independent pathway to *Salmonella typhimurium* invasion gene transcription. *J Bacteriol* 1999; 181: 3096–104.
  27. Stock JB, Surette MB, Levit M, Park P. Two-component signal transduction systems: structure-function relationships and mechanisms of catalysis. In: Hoch JA, Silhavy TJ, eds *Two-component signal transduction*. Washington, DC: ASM Press 1995; 25–51.
  28. Dorman CJ, Chatfield S, Higgins CF, Hayward C, Dougan G. Characterization of porin and *ompR* mutants of a virulent strain of *Salmonella typhimurium*: *ompR* mutants are attenuated in vivo. *Infect Immun* 1989; 57: 2136–40.
  29. Fields PI, Groisman EA, Heffron F. A *Salmonella* locus that controls resistance to antibacterial proteins from phagocytic cells. *Science* 1989; 85: 5189–93.
  30. Miller SI, Kukral AM, Mekalanos JJ. A two-component regulatory system (*phoP phoQ*) controls *Salmonella typhimurium* virulence. *Proc Natl Acad Sci U S A* 1989; 86: 5054–8.
  31. Arricau N, Hermant D, Hervé W, Ecobichon C, Duffrey PS, Popoff MY. The RscB-RcsC regulatory system of *Salmonella typhi* differentially modulates the expression of invasion proteins, flagellin and Vi antigen in response to osmolarity. *Mol Microbiol* 1998; 29: 835–50.
  32. Fugua C, Greenberg EP. Self perception in bacteria: quorum sensing with acetylated homoserine lactones. *Curr Opin Microbiol* 1998; 1: 183–9.
  33. Eichelberg K, Hardt WD, Galán JE. Characterization of SprSA, and AraC-like transcriptional regulator encoded within the *Salmonella typhimurium* pathogenicity island 1. *Mol Microbiol* 1999; 33: 139–52.
  34. Hurme R, Rhen M. Temperature sensing in bacterial gene regulation – what it all boils down to. *Mol Microbiol* 1998; 30: 1–6.
  35. Hurme R, Berndt KD, Normark SJ, Rhen M. A proteinaceous gene regulatory thermometer in *Salmonella*. *Cell* 1997; 55–64.
  36. Pavitt GD, Higgins CF. Chromosomal domains of supercoiling in *Salmonella typhimurium*. *Mol Microbiol* 1993; 10: 685–96.
  37. Jordi BJ, Owen-Hughes TA, Hulton CS, Higgins CF. DNA twist, flexibility and transcription of the osmoregulated *proU* promoter in *Salmonella typhimurium*. *EMBO J* 1995; 14: 5690–700.
  38. Karem K, Foster JW. The influence of DNA topology on the environmental regulation of a pH-regulated locus in *Salmonella typhimurium*. *Mol Microbiol* 1993; 10: 75–86.
  39. Schneider R, Travers A, Kutateladze T, Muskhelishvili G, A DNA architectural protein couples cellular physiology and DNA topology in *Escherichia coli*. *Mol Microbiol* 1999; 34: 953–64.
  40. Falconi M, Colonna B, Prosseda G, Micheli G, Gualerzi CO. Thermoregulation of *Shigella* and *Escherichia coli* EIEC pathogenicity. A temperature-dependent structural transition of DNA modulates accessibility of *virF* promoter to transcriptional repressor H-NS. *EMBO J* 1998; 17: 7033–43.
  41. Leclerc GJ, Tartera C, Metcalf ES. Environmental regulation of *Salmonella typhi* invasion-defective mutants. *Infect Immun* 1998; 66: 682–91.
  42. Drlica K. Control of bacterial DNA supercoiling. *Mol Microbiol* 1992; 6: 425–33.
  43. Azam TA, Ishihama A. Twelve species of the nucleoid-associated protein from *Escherichia coli*. Sequence recognition specificity and DNA binding affinity. *J Biol Chem* 1999; 274: 33105–13.
  44. Droman CJ, Hinton JC, Free A. Domain organization and oligomerization among H-NS-like nucleoid-associated proteins in bacteria. *Trends Microbiol* 1999; 7: 124–8.
  45. Falconi M, Colonna B, Prosseda G, Micheli G, Gualerzi CO. Thermoregulation of *Shigella* and *Escherichia coli* EIEC pathogenicity. A temperature-dependent structural transition of DNA modulates accessibility of *virF* promoter to transcriptional repressor H-NS. *EMBO J* 1998; 17: 7033–43.
  46. O’Byrne CP, Dorman CJ. Transcription of the *Salmonella typhimurium spv* virulence locus is regulated negatively by the nucleoid-associated protein H-NS. *FEMS Microbiol Lett* 1994; 121: 99–105.
  47. Nicholson B, Low D. DNA methylation-dependent regulation of *pef* expression in *Salmonella typhimurium*. *Mol Microbiol* 2000; 35: 728–42.
  48. Ellenberger T, Landy A. A good turn for DNA: the structure of integration host factor bound to DNA. *Structure* 1997; 5: 153–7.
  49. Engelhorn M, Geiselmann J. Maximal transcriptional activation by the IHF protein of *Escherichia coli* depends on optimal DNA bending by the activator. *Mol Microbiol* 1998; 30: 431–41.
  50. Kowarz L, Coynault C, Robbe-Saule V, Norel F. The *Salmonella typhimurium katF* (*rpoS*) gene: cloning, nucleotide sequence, and regulation of *spvR* and *spvABCD* virulence plasmid genes. *J Bacteriol* 1994; 176: 6852–60.
  51. Hengge-Aronis R. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *Escherichia coli*. *Cell* 1993; 72: 165–8.
  52. Lange R, Hengge-Aronis R. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. *Mol Microbiol* 1991; 5: 49–59.
  53. McCann MP, Kidwell JP, Matin A. The putative sigma factor KatF has a central role in development of starvation-mediated general stress resistance in *Escherichia coli*. *J Bacteriol* 1991; 173: 4188–94.
  54. Römling U, Sierralta WD, Eriksson K, Normark S. Multicellular and aggregative behaviour of *Salmonella typhimurium* strains is controlled by mutations in the *agfD* promoter. *Mol Microbiol* 1998; 28: 249–64.
  55. Heiskanen P, Taira S, Rhen M. Role of *rpoS* in the regulation of *Salmonella* plasmid virulence (*spv*) genes. *FEMS Microbiol Lett* 1994; 123: 125–30.
  56. Aviv M, Giladi H, Schreiber G, Oppenheimer AB, Glaser G. Expression of the genes encoding for the *Escherichia coli* integration host factor are controlled by growth phase, *rpoS*,

- ppGpp and autoregulation. *Mol Microbiol* 1994; 14: 1021–31.
57. Heithoff DM, Sinsheimer RL, Low DA, Mahan MJ. An essential role for DNA adenine methylation in bacterial virulence. *Science* 1999; 284: 967–70.
  58. Bearson S, Bearson B, Foster JW. Acid stress responses in enterobacteria. *FEMS Microbiol Lett* 1997; 147: 173–80.
  59. Park YK, Bearson B, Bang SH, Bang IS, Foster JW. Internal pH crisis, lysine decarboxylase and the acid tolerance response of *Salmonella typhimurium*. *Mol Microbiol* 1996; 20: 605–11.
  60. Riesenber-Wilmes MR, Bearson B, Foster JW, Curtis R III. Role of acid tolerance response in virulence of *Salmonella typhimurium*. *Infect Immun* 1996; 64: 1085–92.
  61. Spector MP, Garcia del Portillo F, Bearson SM, Mahmud A, Magut M, Finlay BB, et al. The *rpoS*-dependent starvation-stress response locus *stiA* encodes a nitrate reductase (*narZYWV*) required for carbon-starvation-inducible thermo-tolerance and acid tolerance in *Salmonella typhimurium*. *Microbiology* 1999; 145: 3035–45.
  62. Gahan CG, Hill C. The relationship between acid stress responses and virulence in *Salmonella typhimurium* and *Listeria monocytogenes*. *Int J Food Microbiol* 1999; 50: 93–100.
  63. Abshire KZ, Neidhardt FC. Analysis of proteins synthesized by *Salmonella typhimurium* during growth within host macrophage. *J Bacteriol* 1993; 175: 3734–43.
  64. Hall HK, Foster JW. The role of *fur* in the acid tolerance response of *Salmonella typhimurium* is physiologically and genetically separable from its role in iron acquisition. *J Bacteriol* 1996; 178: 5683–91.
  65. Bang IS, Kim BH, Foster JW, Park YK. OmpR regulates the stationary-phase acid tolerance response of *Salmonella enterica* serovar Typhimurium. *J Bacteriol* 2000; 182: 2245–52.
  66. Foster JW. *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. *J Bacteriol* 1991; 173: 6896–902.
  67. Foster JW. When protons attack; microbial strategies of acid adaptation. *Curr Opin Microbiol* 1999; 2: 170–4.
  68. Lee IS, Lin J, Hall HK, Bearson B, Foster JW. The stationary-phase sigma factor sigma S (RpoS) is required for sustained acid tolerance response in virulent *Salmonella typhimurium*. *Mol Microbiol* 1995; 17: 155–67.
  69. Lee IS, Slonczewski L, Foster JW. A low-pH-inducible stationary-phase acid tolerance response in *Salmonella typhimurium*. *J Bacteriol* 1994; 176: 1422–6.
  70. Bang IS, Kim BH, Foster JW, Park YK. OmpR regulates the stationary-phase acid tolerance response of *Salmonella enterica* serovar Typhimurium. *J Bacteriol* 2000; 182: 2245–52.
  71. Bearson BL, Wilson L, Foster JW. A low pH-inducible PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress. *J Bacteriol* 1998; 180: 2409–17.
  72. Sukupolvi S, Lorenz RG, Gordon JI, Bian Z, Pfeifer JD, Normark SJ, et al. Expression of thin aggregative fimbriae promotes interaction of *Salmonella typhimurium* SR-11 with mouse small intestinal epithelial cells. *Infect Immun* 1997; 65: 5320–5.
  73. van der Velden AW, Bäumlér AJ, Tsolis RM, Heffron F. Multiple fimbrial adhesins are required for full virulence of *Salmonella typhimurium* in mice. *Infect Immun* 1998; 66: 2803–8.
  74. Galán JE. Molecular genetic bases of *Salmonella* entry into host cells. *Mol Microbiol* 1996; 20: 263–71.
  75. Galán JE, Collmer A. Type III secretion machineries: bacterial devices for protein delivery into host cells. *Science* 1999; 284: 1322–8.
  76. Pegues DA, Hantam MJ, Behlau I, Miller SI. PhoP/PhoQ transcriptional repression of *Salmonella typhimurium* invasion genes: evidence for a role in protein secretion. *Mol Microbiol* 1995; 17: 169–81.
  77. García Vescovi E, Soncini FC, Groisman EA. Mg<sup>2+</sup> as an extracellular signal: environmental regulation of *Salmonella* virulence. *Cell* 1996; 84: 165–74.
  78. Gunn JS, Hohmann EL, Miller SI. Transcriptional regulation of *Salmonella* virulence: a PhoQ periplasmic domain mutation results in increased net phosphotransfer to PhoP. *J Bacteriol* 1996; 178: 6369–73.
  79. 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: 715–27.
  80. Rakeman JL, Bonifield HR, Miller SI. A HilA-independent pathway to *Salmonella typhimurium* invasion gene transcription. *J Bacteriol* 1999; 181: 3096–104.
  81. Rhen M, Riikonen P, Taira S. Transcriptional regulation of *Salmonella enterica* virulence plasmid genes in cultured macrophages. *Mol Microbiol* 1993; 10: 45–56.
  82. Marshall DG, Sheehan BJ, Dorman CJ. A role for the leucine-response regulatory protein and integration host factor in the regulation of the *Salmonella* plasmid virulence (*spv*) locus in *Salmonella typhimurium*. *Mol Microbiol* 1999; 34: 134–45.
  83. Cunnig C, Brown L, Elliott T. Promoter substitution and deletion analysis of upstream region required for *rpoS* translational regulation. *J Bacteriol* 1998; 17: 4564–70.
  84. Altier C, Suymoto M, Ruiz AI, Burnham KD, Maurer R. Characterization of two novel regulatory genes affecting *Salmonella* invasion gene expression. *Mol Microbiol* 2000; 35: 635–46.
  85. Luccini S, Thompson A, Hinton JCD. Microarrays for microbiologists. *Microbiology* (in press).