

Perspectives Series: Host/Pathogen Interactions

Regulation of Bacterial Virulence Gene Expression by the Host Environment

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Bacterial pathogens must adapt to a wide variety of changing environmental conditions during the infection cycle. Upon encountering the host, pathogens must express specific gene products to persist and proliferate in the appropriate location and to circumvent host defenses. During the course of the infection, the host environment may change markedly. All pathogens must also ensure successful transmission, which may entail prolonged periods in external environments or adaptation to an intermediate host of a different species. All bacteria regulate gene expression in response to different environmental signals, a property that is crucial to their ability to compete with other organisms. In general, gene products that confer a growth or survival advantage in a particular situation are expressed, while unnecessary functions are downregulated. The same principle applies to genes encoding virulence traits in pathogens. These virulence genes are subject to complex regulatory mechanisms that ensure expression in the appropriate host environment.

Since regulation of virulence genes is a common property of pathogens, strong selective pressures must operate against simple constitutive expression of virulence traits. The general metabolic economy of the bacterial cell selects against unnecessary expenditure of energy. However, there are likely to be specific reasons that a pathogen does not display all of its virulence features simultaneously and continuously. Surface molecules that localize the bacteria to a specific site in the host have to be altered if the organism is to change its location or be transmitted to a new host. Invasive pathogens need one set of genes to gain access to the host and a different set to proliferate and circumvent host defenses in the tissues. Certain key virulence structures may become a liability for the organism as antigens when the host mounts an immune response. Some pathogens can downregulate or switch antigens during the course of infection. To survive in the environment during transmission, different genes may be important, and for those organisms that infect an intermediate host, an entire new set of virulence genes is required.

Despite the complex regulatory requirements of bacterial pathogens, the environmental cues that act as signals for the control of virulence gene expression in animal pathogens are generally simple physical and chemical factors: temperature,

osmolarity, O₂, CO₂, pH, reactive oxygen and nitrogen compounds, nutrient availability, and inorganic ion concentrations. Many of these factors change in the transitions from the external environment to the host, between different locations in the host, and between different hosts. Bacteria are able to respond to a complex environment by integrating the signals from a variety of these cues to sense their location and express the appropriate genes.

Temperature is a particularly useful signal for mammalian pathogens since the host temperature is relatively constant and elevated with respect to the ambient environment. Temperature-sensitive gene expression allows the organism to discriminate between the host and the outside world, and certain genes required to produce disease, such as the invasion genes of *Shigella* and the plasmid genes encoding secreted proteins of *Yersinia*, are not expressed at 25°C but are induced at 37°C (1, 2). Elevations in temperature also induce the heat shock stress response, a prominent regulatory circuit important for many pathogens.

While simple chemical cues are not unique to the host, they can be used by the bacteria to discriminate between different locations. Osmolarity varies in the gastrointestinal tract and tissue osmolarity is higher than fresh water environments. High osmolarity stimulates *Salmonella* invasion and the osmosensor, OmpR, is essential for virulence in several bacterial species (3, 4). O₂ concentrations vary greatly between the lumen of the bowel and perfused tissue, and low oxygen stimulates *Salmonella* invasion from the gastrointestinal tract. CO₂ concentration of tissues and the respiratory mucosa is elevated with respect to the normal atmospheric level, and CO₂ has been shown to regulate M-protein synthesis in streptococci (5). pH changes abruptly in the intestinal tract and regulates synthesis of acid stress proteins in bacteria exposed to gastric contents or signals localization in the small bowel by the increase in pH. Invasive pathogens are exposed to the toxic effects of reactive oxygen and nitrogen intermediates produced by phagocytic cells, inducing crucial resistance and repair mechanisms (6). Lack of nutrients induces a global stress response in bacteria that regulates virulence gene expression in *Salmonella* and also is likely to be important for survival in hostile external environments during transmission of pathogens (7). Inorganic ion concentrations are used by pathogenic bacteria to sense their location. Iron plays a key regulatory role, since virtually all available iron on mucosal surfaces and in the tissues is bound to host proteins (8). Low iron induces a variety of virulence traits including toxin production in *Corynebacterium diphtheriae* and synthesis of a specialized siderophore, aerobactin, in uropathogenic *Escherichia coli*. Low Mg²⁺ in the macrophage phagosome is sensed by the two-component regulatory system PhoPQ of *Salmonella*, leading to induction of genes required for intracellular survival (9).

In contrast to mammalian pathogens, virulence gene regu-

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latory mechanisms involving host-pathogen and pathogen-pathogen communication mediated by more specific diffusible molecules are commonly found in bacteria that infect plants. The virulence genes of *Agrobacterium tumefaciens*, the cause of crown gall tumors, are controlled by a two-component regulatory system that responds to the presence of phenolic compounds produced by plants. The system ensures that the virulence genes are expressed when the bacteria are in close proximity to the plant. This precedent should stimulate a search in animal pathogens for similar systems that respond to specific products made by the host. Certain plant pathogens of the genera *Agrobacterium*, *Erwinia*, and *Pseudomonas* use a form of communication between bacterial cells termed "quorum sensing" (10). Bacteria synthesize acylated derivatives of homoserine lactone which accumulate in the extracellular environment at levels related to the cell density of the bacteria. When a certain density is reached, the acylated homoserine lactone induces synthesis of the target genes in the entire bacterial population. This system is also used by the symbiotic light-emitting bacteria that infect certain deep sea fish. Although this quorum sensing mechanism mediated by acylated homoserine lactone has not yet been shown to regulate virulence in primary, nonopportunistic pathogens of higher vertebrates, cell density-dependent regulation based on an octapeptide pheromone has been reported recently in *Staphylococcus aureus* (11). The pheromone accumulates in the post-exponential phase of growth, signaling the cells to express secreted virulence proteins and repressing the production of surface proteins. Additional quorum sensing regulatory systems are likely to be found in animal pathogens for which colonization with a certain inoculum precedes disease.

These conditions that control virulence gene expression in pathogens also stimulate global regulatory changes in many genes common to both pathogens and commensal bacteria. Therefore, virulence gene expression must be integrated with regulatory circuits for a variety of "housekeeping" genes. During evolution, pathogens have faced a genetic problem with this regulatory integration since virulence genes have been acquired frequently on accessory genetic elements, such as plasmids and phage, or as blocks of genetic information inserted into the chromosome as pathogenicity islands. Integration with the global bacterial regulatory circuits often involves an adapter mechanism in which the virulence gene cluster encodes its own regulatory element, but this element also responds to selected global control signals of the organism. In this manner, virulence genes that are specific for a pathogen are found to be regulated by control elements that are widespread among both pathogens and nonpathogens. Thus, virulence genes have adopted the specific bacterial signal transduction systems that respond to the appropriate environmental cues and ensure optimal virulence gene expression at the proper time during infection.

Salmonella species are facultative intracellular pathogens that illustrate many of these principles of virulence gene regulation during pathogenesis. *Salmonella* infection is acquired by ingestion and the organisms must colonize and invade the small bowel mucosa to initiate disease. This first stage in pathogenesis is dependent on a 40-kb block of genes comprising the *Salmonella* pathogenicity island 1 (SPI1),¹ located at

centisome 63 on the chromosome (3). The expression of these invasion genes is regulated in response to environmental cues present in the small bowel. To cause systemic disease, nontyphoid *Salmonella* serovars must survive and replicate inside host cells, probably primarily within macrophages (12). In this second stage of pathogenesis, regulatory mechanisms signal the induction of specific chromosomal and plasmid-encoded virulence genes required for survival and growth inside the phagocytic vacuole. At both stages of the infection, *Salmonella* gene expression involves both virulence-specific gene products as well as global bacterial regulatory mechanisms.

The regulation of *Salmonella* invasion of epithelial cells is depicted in Fig. 1. Efficient entry of *Salmonella* into epithelial cells requires production of secreted invasion proteins (Sips) which act both on the epithelial cell surface and internally to remodel the cell membrane into ruffles, leading eventually to macropinocytosis of the bacteria (3, 13, 14). The Sips are exported from the bacteria by a type III contact-dependent protein secretory system. The secretion of the Sips is stimulated by contact between the bacteria and the epithelial cell membrane (3). Both the Sips and the type III system are encoded by the *Salmonella* pathogenicity island 1, and expression of these genes is coordinately regulated by the *hilA* gene product (15).

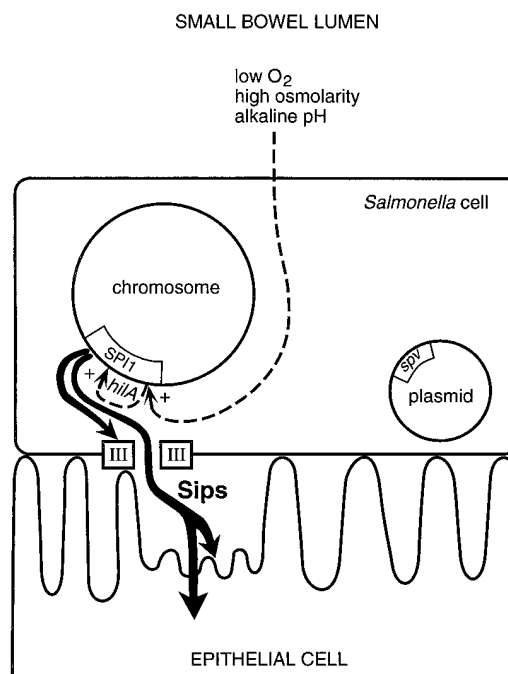


Figure 1. Model for the regulation of *Salmonella* invasion genes during intestinal infection. A *Salmonella* cell is depicted in association with an intestinal epithelial cell in the small bowel. Exposure to environmental cues present in the bowel lumen, including low O₂ concentrations, relatively high osmolarity, and alkaline pH, acts to induce expression of the *hilA* gene located on SPI1. HilA in turn induces operons in SPI1 encoding the components of a type III protein secretion system as well as the secreted proteins (Sips). Contact between the bacterium and the epithelial cell stimulates Sip secretion by the type III system located at the bacterial cell surface. The Sips interact with the epithelial cell membrane and certain secreted proteins enter the cytoplasm. The Sips promote uptake of the bacterium into a membrane-bound intracellular vacuole. The virulence plasmid *spv* genes are not required in this stage of pathogenesis.

1. Abbreviations used in this paper: pags, PhoP-activated genes; Sips, secreted invasion proteins; SPI1, *Salmonella* pathogenicity island 1.

The HilA protein activates transcription of several operons within the pathogenicity island. *hilA* itself is also located in the SPI1 and appears to be a unique and specific regulator for the *Salmonella* invasion genes. *hilA* expression increases in response to the environmental cues that lead to invasion gene expression: low O₂ levels, relatively high osmolarity, and alkaline pH, conditions present in the lumen of the small bowel. The mechanisms by which these external conditions regulate *hilA* expression are unknown, but recent work indicates that *hilA* is controlled in part through a common sensory transduction circuit not specific for *Salmonella*. The *sirA* gene, located outside the pathogenicity island and allelic with the *E. coli* *uvrY* gene, is required for efficient *hilA* expression (16). The sequence of SirA indicates that it is a transcriptional response regulator of a two-component regulatory system, suggesting a role in the transduction of environmental signals for the regulation of *hilA* expression. For invasion gene expression, HilA appears to be the adapter that integrates regulatory signals from the bacterial cell to produce a coordinate response in expression of the invasion locus.

In the second stage of *Salmonella* pathogenesis, the organisms are taken up by macrophages in the bowel, located primarily in Peyer's patches, and later disseminate to macrophages in systemic organs. Survival of *Salmonella* within macrophages is an essential virulence phenotype (12). Inside the macro-

phage, *Salmonella* proliferate in the membrane-bound phagocytic vacuole, and numerous genes are required for survival and growth in this environment. Two regulatory circuits that control virulence gene induction in the intracellular environment of macrophages have been characterized in detail: one mediated by the alternate sigma factor σ^s (RpoS) and the other involving the two-component regulatory system PhoPQ (Fig. 2). After uptake of *Salmonella* by macrophages in vitro, there is a lag period of up to several hours in which little or no net growth of the bacteria occurs. This limitation of growth appears to be an early signal sensed by the bacteria soon after phagocytosis and leads to rapid induction of the *rpoS* gene on the chromosome, encoding an alternative sigma factor for the bacterial RNA polymerase (17). σ^s controls a global stress response that has been found widely distributed among gram-negative bacteria. Growth limitation, mediated by a variety of adverse conditions including nutrient starvation, high osmolarity, and low pH, signals the σ^s stress response. *rpoS* expression is regulated by complex mechanisms operating at the levels of transcription, translation, and protein stability. Recent evidence suggests that a two-component signal transduction system including the response regulator *rssB* controls σ^s levels by regulating the activity of the ClpXp protease involved in σ^s degradation (18). Increased levels of σ^s in the bacterial cell cause global changes in gene expression due to replacement of the vegetative σ^{70} with σ^s in the RNA polymerase holoenzyme. Expression of σ^s -dependent genes increases while the transcription of a variety of other genes decreases. The σ^s -dependent stress response renders *Salmonella* more resistant to oxidative agents, DNA damage, high osmolarity, acid pH, and prolonged starvation (7).

In *Salmonella*, σ^s is also required for the expression of the plasmid-encoded *spv* genes (7). Virulence plasmids carrying the *spv* genes are found in certain nontyphoid *Salmonella* serovars that are highly associated with severe systemic disease, such as *S. choleraesuis*, *S. dublin*, and *S. typhimurium* (19). The *spv* genes greatly enhance the ability of these *Salmonella* strains to proliferate at extraintestinal sites, most likely within tissue macrophages. The *spv* locus consists of five genes, designated *spvRABCD*, and is highly conserved among *Salmonella* serovars. *SpvR* encodes a transcriptional activator which positively regulates its own synthesis and is required for expression of the *spvABCD* operon. SpvR binds to both the *spvR* and *spvA* promoters (20). However, efficient transcription from both *spv* promoters requires σ^s , and its level within the bacterial cell regulates expression of both *spvR* and the *spvABCD* operon (21). For the *spv* genes, SpvR is the adapter that links specific expression of the *spv* locus to the general bacterial stress response mediated by σ^s .

σ^s levels, as measured by an RpoS::LacZ protein fusion, increase rapidly in the first hour after ingestion of *S. typhimurium* by macrophages (17). This increase in σ^s induces *spvR*, and positive autoregulation of *spvR* ensures a rapid increase in SpvR levels in response to the σ^s signal. SpvR and σ^s act in concert at the *spvA* promoter to induce expression of the *spvABCD* genes, leading to sustained synthesis of the Spv proteins for up to 6 h after uptake of the bacteria by macrophages. The Spv proteins appear to act by an unknown mechanism to increase proliferation of the bacteria in the intracellular environment (Libby, S., L. Adams, T. Ficht, C. Allen, H. Whitford, N. Buchmeier, S. Bossie, and D. Guiney, manuscript submitted for publication).

A second regulatory circuit consisting of the two-compo-

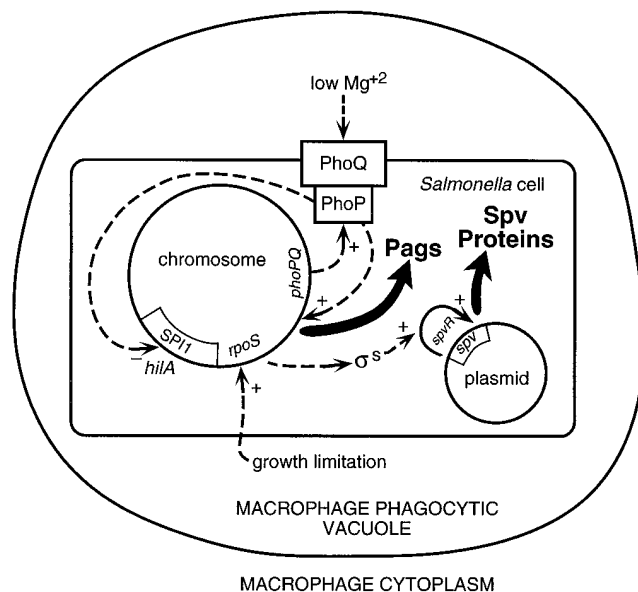


Figure 2. Model for the regulation of *Salmonella* virulence genes involved in the extraintestinal infection of tissue macrophages. After phagocytosis, *Salmonella* cells remain within the phagocytic vacuole. This environment limits the growth of the organism, leading to increased levels of the alternative sigma factor σ^s , the product of the chromosomal *rpoS* gene. σ^s increases synthesis of the transcriptional activator SpvR which acts together with σ^s to induce the *spv* operon on the virulence plasmid. The Spv proteins appear to enhance proliferation of the *Salmonella* in the intracellular environment. During maturation of the phagocytic vacuole, low Mg²⁺ levels activate the two-component PhoPQ regulatory system, leading to synthesis of several proteins designated Pags, the products of PhoP-activated genes. The Pags appear to be involved in survival of the bacteria inside macrophages. PhoP also negatively regulates *hilA* and inhibits production of invasion gene products.

nent signal transducer PhoPQ controls the expression of additional *Salmonella* virulence genes at a later time after phagocytosis (22). PhoQ responds to low Mg^{2+} levels which appear to be present as the phagosome is processed in the macrophage (9). PhoQ activates the response regulator PhoP by phosphorylation, leading to induction of PhoP-activated genes (*pags*) by 3 h after phagocytosis. *phoP* is required for virulence (23, 24), and production of the Pag proteins is likely to be essential for long-term survival of the organism in the macrophage. In addition to gene induction, PhoP also represses the synthesis of other loci including *hilA*, the regulator of the invasion genes (15). This finding suggests that the intracellular environment downregulates invasion gene expression, consistent with the demonstration that the invasion genes are not required during the extraintestinal phase of *Salmonella* pathogenesis (25).

The expression of the *Salmonella* invasion, *spv*, and *pag* loci in response to specific host environments illustrates the genetic mechanisms for integrating virulence gene control into general bacterial signal transduction and stress response regulatory circuits. Two-component regulatory systems play prominent roles in the ability of the bacteria to sense and respond to host environments in both intestinal and systemic phases of *Salmonella* infection. Specific adapter regulatory molecules, such as HilA and SpvR, are induced by general signal transduction pathways triggered by environmental cues. HilA and SpvR then act to drive transcription of specific virulence genes required at different stages of pathogenesis.

It is remarkable that pathogens can use a limited repertoire of nonspecific environmental factors to accurately sense their location in the host and express the appropriate virulence genes. However, in addition to simple physical and chemical factors in the host, recent evidence suggests that contact between the bacterial pathogen and the host cell has an important regulatory role in the pathogenesis of infection. The host cell attachment of *E. coli* mediated by P-pili in the urinary tract induces transcription of the *airS* gene encoding a sensor-activator protein involved in the control of iron-acquisition systems (26). Protein secretion stimulated by contact with host cells (mediated by type III systems) is essential for the virulence of several genera, including *Salmonella*, *Shigella*, and *Yersinia* (3). These systems provide a mechanism for the bacteria to sense contact with the host and respond by increasing virulence gene expression or stimulating the delivery of preformed virulence proteins to the host cell. Continued work in this field will elucidate the complex interactions between global regulatory mechanisms and more specific sensory systems that are likely to account for differential virulence gene expression in distinct host environments.

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