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A Tale of 6 Sigmas: How Changing Partners Allows *Salmonella* to Thrive in the Best of Times and Survive the Worst of Times

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1. Introduction

Salmonella enterica are rod-shaped, facultative anaerobic, Gram-negative members of the Enterobacteriacae family (Dougan et al., 2011). Most people have heard of the bacteria and generally associate it with food-borne illness. Despite general public knowledge of the health risks associated with and precautions taken to prevent its spread, *Salmonella* continues to cause many problems. One approach toward curbing this spread and reducing the negative impact of *S. enterica* could be genetic analysis, with an ultimate goal of understanding why the bacteria are able to survive attempts to destroy them.

It has been suggested that the *Salmonella* genus diverged from *Escherichia coli* somewhere between 100 and 150 million years ago (Dougan et al., 2011). While there is evoluntionary distance between the two genera, much of the genetic information has been conserved, and as a result, the study of one organism has provided insight into the study of the other. *Salmonella* spp. are generally considered to be pathogenic and can have both warm- and cold-blooded hosts (Dougan et al., 2011). More recent evolution has occurred within the *Salmonella* genus itself. *Salmonella enterica* has evolved into many different subspecies and serovars that manifest in dramatically different ways across a variety of hosts despite sharing 95% of the same genetic information (McDermott et al., 2011). From a medical perspective, *Salmonella* genetics are particularly important. Although a single-celled organism, due to its long evolutionary history with humans and other organisms, these bacteria has developed several sophisticated mechanisms to survive the immune systems of its hosts and evade sanitation efforts to kill it. Understanding how this survival at the most fundamental of levels, it may be possible to more specifically combat the bacteria.

Salmonella typically reach their hosts through the consumption of contaminated food or water. Once inside its host, the bacteria must persist through various levels of pH, temperature, osmolarity, and nutrient availability (Ohl & Miller, 2001). The pathogen must also face various attempts by the host's immune system to eliminate it. Each different environment and each assault on the bacteria's integrity must be addressed by the organism in order to survive. The ability of the organism to thrive in a multitude of environments and persist to establish infection in its host is governed by the expression of different genes.

While there are a multitude of regulatory pathways within *Salmonella* that can influence gene expression, one of the most fundamental comes from the usage of alternate sigma factors by the cell's RNA polymerase, as is the case for most prokaryotes. Sigma factors facilitate differential gene expression by reversibly binding to the RNA polymerase core enzyme and providing specificity for certain promoter regions. The various sigma factors have different affinities for particular promoters as well as for the core enzyme itself. Similar to other cellular proteins, sigma factors are regulated at a variety of levels. Transcription in *Salmonella*, as in all prokaryotes, requires a sigma factor, and ultimately all gene expression is affected by sigma factors and their activity.

Sigma factors were originally discovered as protein factors that stimulate RNA synthesis from DNA using DNA-dependent RNA polymerase (Burgess & Travers, 1969). These proteins all share four regions of similarity indicative of a common function (Kutasake et al., 1994). For the group of closely related sigma factors, special regions within the protein recognize specific regions of the DNA as promoters versus non-promoter regions of DNA (Dombroski et al., 1992). These DNA regions include conserved sequences centered around the -35 and -10 positions with respect to the transcription initiation site. By truncating the sigma protein at various locations, researchers were able to determine that four conserved regions of the sigma factors were responsible for locating different areas of the promoter region. For example, region 4 of the sigma factor is found to recognize the consensus sequence around -35, while regions 2 and 3 recognize the -10 consensus sequence (Dombroski et al., 1992). Region 1 of the sigma factor, the amino terminus of the protein, blocks regions 2, 3, and 4 from interacting with the DNA (Dombroski et al., 1993). Binding of the sigma factor to the core enzyme blocks region 1 and allows interaction of the other three regions with the DNA (Dombroski et al., 1992). In this way, the sigma factor cannot interact with DNA without being bound by RNA polymerase. While it was understood that a sigma factor was necessary to facilitate transcription, their power to regulate gene expression was not fully understood.

2. Early virulence-related genetic studies

As with most pathogenic microorganisms, early genetic research focused on the disease-causing properties of *Salmonella*. Preliminary studies involving virulence properties of *Samonella* revealed that in the absence of a functional copy of several genes, the bacteria was unable to survive to cause infection inside its host. Further studies of each of these genes revealed that while all of the genes were required for optimal virulence, the gene expression was not under the same regulatory control. Baumler and his colleagues examined nearly 30 mutant strains of *Salmonella* Typhimurium that had shown attenuated ability to infect and survive inside mouse macrophages (Baumler et al., 1994). Baumler concluded that these genes all made contributions to the virulence properties of *Salmonella*.

Some of the particular genes that Baumler concluded were disrupted in the attenuated strains were *purD*, *prc*, *fliD*, and *nagA* (Baumler et al., 1994). Other researchers have examined the transcriptional control of these genes to understand why they are so essential to the virulence capabilities of *Salmonella*.

As many sigma factors are closely related, there is a high degree of homology between their structures and therefore promoter affinities. However, as few as one or two base pair change

can dramatically change which sigma factor recognizes the promoter (Römling et al., 1998). The *purD* gene encodes 5'-phosphoribosylglycinamide synthetase, which is involved in purine nucleotide synthesis (Aiba & Mizobuchi, 1989). While these genes have easily identifiable -10 consensus sequences, none appear to have the -35 region similar to those typically recognized by the primary sigma factor (Kilstrup et al., 1998). Potentially, the ambiguity of the promoter region shows the ability to be used by multiple sigma factors.

A second gene, *prc*, encodes a protease that in closely related organisms has been found to play a role in response to cell wall stress (Wood et al., 2006). In these organisms, *prc* is preceded by a consensus sequence for a sigma factor showing a great deal of similarity to the sigma factor in *E. coli* and *Salmonella* that responds to a variety of global stresses, including damage to the cellular envelope (Wood et al., 2006).

The *fliD* gene encodes part of the flagella filament, needed for the motility of the bacteria (Kutsukake et al., 1994). This gene is proceeded by a consensus sequence that can only be used by the flagella-specific sigma factor (Kutsukake et al., 1994) and is part of a highly temporally and spatially regulated pathway that ensures flagella are expressed readily in times that motility is necessary and repressed when the bacteria have not formed the appropriate primary structures for the flagellar.

The *nagA* gene product is N-acetylglucosamine-6-phosphate deacetylase in *E. coli* and has the same function in *Salmonella* Typhimurium (Baumler et al., 1994). This gene was found to have consensus sequence in the -10 region requiring the activation of a magnesium sensitive regulator in the presence of the housekeeping sigma (Minagawa et al., 2003). Based only on the extracellular availability of magnesium, the primary sigma factor is responsible for the transcription of the gene, provided a secondary regulatory system is activated.

With the genes that Baumler examined, in combination with other research indicating that each of these types of genes was under different regulatory control by particular sigma factors, a pattern began to emerge. Genes responsible for the organism's response to particular threats to its integrity were under the transcriptional direction of particular sigma factors. The importance of sigma factors as transcriptional regulators is further revealed by their stability over time (Sutton et al., 2000) and the high degree of homology between closely related species (Guiney et al., 1995).

3. A tale of six sigmas

To date, six different sigma factors have been discovered to be encoded within the *Salmonella* genome that are responsible for transcription from a variety of promoters in response to different phases of the organism's life as well as environmental conditions. Acting together in a complex, as an interconnected web of gene regulation, they enable *Salmonella* to withstand and thrive inside infected hosts.

Sigma factors were characterized as proteins before their function as essential elements of the holoenzyme became clear. As such, each sigma factor is known by a variety of names. Designations with *rpo* or Rpo are used across species and refer to the particular stress to which the sigma factor responds. A more contemporary convention is to use a lower case Greek sigma with the molecular weight of the sigma factor as a superscript. In this text, all molecular weights refer to those found in *Salmonella* and *E. coli.*

Most of these proteins, σ^{70}, σ^{E}, σ^{H}, σ^{S}, and σ^{F}, belong to the same family of sigma factors, potentially all derived from some ancestral form or ancestral regulatory process. The other sigma factor, σ^{N}, belongs to a different family, although it is the only modern day example found, and may belong to a more ancient regulatory system that has become obsolete with current patterns of growth and reproduction for bacteria like *Salmonella*. While the housekeeping sigma was found to facilitate most gene expression during exponential growth, each of the other sigma factors was found to help the organism address different environmental stresses. Each sigma factor recognizes a different consensus sequence within the promoter region. The relative affinities of multiple sigma factors for the same promoter region determine which recognizes it more often at a specific intracellular concentration.

3.1 σ^{N} – Nitrogen regulation

σ^{N} seems to be more evolutionarily distant from the other alternate sigma factors and it may be the remnants of a more ancient regulatory system. In fact, the processes governed by σ^{N} may not be essential or may be under transcriptional control of another sigma factor (Morett & Segovia, 1993). These processes include nitrogen fixation, dicarboxylic acid transport, and hydrogen oxidation (Morett & Segovia, 1993). Down-regulating expression from RpoN-dependent genes provides increased resistance to killing by host cationic antimicrobial peptides (Barchiesi et al., 2009), indicating that some of these processes may even be detrimental to the organism in certain conditions. In some related species σ^{N} is related to pathogenicity, but that does not appear to be in *Salmonella* (Studholme, 2002).

The differences between σ^{N} and the rest of the sigma factors are profound. There is almost no sequence similarity between the *rpoN* gene and genes for other known sigma factors, also suggesting a different origin (Morett & Segovia, 1993). σ^{N} promoters are unique in that they have conserved consensus sequences centered at -24 and -12 nucleotides from the transcription start site, as opposed to -35 and -10 (Barrios et al., 1999). A highly conserved RpoN-Box is involved in the recognition of the -24 and -12 DNA sequences (Barrios et al., 1999). The distance between the -24 and -12 elements is more stringent than the analogous distance between the -35 and -10 elements for the σ^{70} family of sigma factors, indicating a highly controlled regulation (Barrios et al., 1999). Moreover, the sequences at the -24 and -12 elements have highly conserved GG and GC regions respectively, also suggesting a high level of regulatory control (Barrios et al., 1999).

While the σ^{N} protein is very different from other alternate sigma factors, the interaction between the sigma factor and template DNA is also distinct. The σ^{70} family of sigma factors do not form stable closed complexes and transcription will start spontaneously (Barrios et al., 1999). Unlike other sigma factors, the σ^{N} and core enzyme form a stable closed complex. In this way, σ^{N} binding to the core enzyme actually blocks transcription because the open complex must be activated (Buck & Cannon, 1992). The binding of the RNA polymerase holoenzyme with σ^{N} as the sigma factor cannot induce DNA melting alone, similar to the RNA polymerase II system in eukaryotes (Buck et al., 2000). σ^{N} may bind to DNA first rather than binding to the core enzyme first (Buck et al., 2000). This is supported by the fact that σ^{N} binds to a different location on the core enzyme than σ^{70} and in doing so may be able to assist in DNA melting once activated (Buck et al., 2000).

Because it forms a stable closed complex, the RNA polymerase with σ^N as the sigma factor requires enhancer proteins for activation. Each enhancer protein is under the regulation of its own signal transduction pathway, allowing response to various environmental conditions (Buck et al., 2000). All the enhancer proteins have hidden ATPase activity that allows for the DNA melting necessary to initiate transcription (Buck et al., 2000).

3.2 The housekeeping sigma σ^{70}

The other five sigma factors appear to be evolutionarily related, developing from the original or primary sigma factor. RpoD or σ^{70} is the housekeeping sigma factor and is responsible for the transcription of most of the genes in bacterial cells growing exponentially (Ishihama, 1993). When *rpoD* was found in the genome for *E. coli*, it was determined that the gene sequence had a high degree of homology between other *rpoD* genes from closely related species (Scaife et al., 1979). Further genomic analysis determined that *rpoD* is found in a transcript with the 30S ribosomal protein S21 and DNA primase (Burton et al., 1983). This operon was the first discovered operon containing proteins involved in transcription, translation, and replication (Burton et al., 1983). $E\sigma^{70}$ (the holoenzyme containing the core enzyme associated with σ^{70}) does not form a stable closed complex and transcription begins spontaneously (Barrios et al., 1999), requiring no enhancer proteins. Moreover, the σ^{70} concentration found inside a cell undergoing exponential growth is less than the concentration of core enzymes, indicating the level of the sigma factor present may regulate the level of transcription (Burton et al., 1983).

3.3 σ^E – Response to extracytoplasmic stress

When the bacteria face stressors, other sigma factors are involved in the expression of genes necessary to survive the stress, such as σ^E, σ^{24}, or RpoE which results in transcription of genes to combat envelop stress (Kenyon et al., 2005). RpoE is constitutively expressed in the bacteria, held inactive by interaction with various binding proteins. The *rpoE* gene seems to be the most highly conserved of alternate sigma factors across several species, as are the genes under its transcriptional control.

RpoE must be able to respond to a signal coming from outside of the cell, while the protein itself resides inside the bacterium. It appears that a transmembrane protein, RseA, interacts with RseB on the periplasmic side and with σ^E on the cytoplasmic side. An area of the DegS protein on the periplasmic side recognizes unfolded proteins resulting in proteolysis of the periplasmic side of RseA. Cleaved RseA is a target for RseP, which then cleaves the transmembrane portion of RseA, releasing the RseA/ σ^E complex from the membrane and the unstable cytoplasmic portion of RseA is quickly degraded by cytoplasmic proteases (Muller et al., 2009). RseB also interacts on the periplasmic side with both DegS and RseP to control the activity of these proteases in the absence of a stress response (Muller et al., 2009). The strength of the signal is directly proportional to the number of misfolded outer membrane proteins.

While response to envelop stress is typically the signal necessary to release RpoE from RseA, acid stress may also result in the same response. It was found that mutants deficient in RpoE activity showed increased susceptibility to acid and reduced ability to survive inside macrophages. The RseP domain was required for this response to the acid shock, but its proteolytic activity was not dependent on DegS (Muller et al., 2009). It is proposed that the

acidic milieu affects the interaction between RseB and RseP, which normally keeps RseP inactive, so that RseP is released to act on RseA, discontinuing negative control over σ^E (Muller et al., 2009). Both DegS and RseP have cytoplasmic and periplasmic domains, and the acid response appears to be independent of the envelope stress response. Again, the response strength is contingent upon the acidity of conditions and the length of exposure.

Once σ^E is released to interact with RNA polymerase, not all σ^E - dependent genes are transcribed equally. Within the approximately 60 promoters examined that required σ^E for transcription, there were few very strong promoters (showing high affinity) but many relatively weak promoters. The strong promoters were conserved across both *E. coli* and *S. enterica*, and were typically involved in maintaining porin homeostasis (Mutalik et al., 2009). Varying strength of promoters allow quick and efficient adaptation to different environments by being able to transcribe different genes in response to various signals (Mutalik et al., 2009). If the stress signal is strong, the cellular concentration of σ^E will increase to transcribe at high rates from weak promoters.

In order to prevent wasted energy and further damage to the cell, the activation of σ^E also results in the down-regulation of *omp* (outer membrane protein) mRNA (Papenfort et al., 2006). The cell also prevents these nascent mRNAs from producing misfolded proteins while avoiding destruction by the exocytoplasmic stress. Two small non-coding RNAs, RybB and MicA, not under the control of RpoE, collectively expedite the destruction of *omp* mRNAs. Under normal conditions, the cellular machinery making OMPs is still not perfect and some misfolded proteins are generated. In this case, the same two sRNAs are involved in the response to fix the problem by inducing the σ^E response, but at a much lower level than would be found in bacteria responding to prolonged stress (Papenfort et al., 2006). As such, the two sRNAs are most likely under the transcriptional control of the housekeeping sigma factor and their increased activity helps to induce σ^E activity.

As far as specific genes governed by σ^E, the parts of the σ^E regulon that are highly conserved across species are involved in making the cell wall and outer membrane of Gram-negative bacteria (Rhodius et al., 2006). The variable portion may be involved in the alternative lifestyles that the studied species utilize. A genome-wide search was done for σ^E-dependent genes in several species including *E. coli* and *Salmonella* Typhimurium, determining that several genes were at the core of the σ^E regulon. Some genes were involved in making lipoproteins, such as *yfiO*, *yeaY*, and *yraP*. Others were involved in outer membrane protein synthesis and modification, like *yeaT*, *skp*, *fkpA*, and *degP*. And still others were involved in cell envelope structure, such as *plsB*, *bacA*, *ahpF*, and *ygiM*. Interestingly, both *rpoE* and *rpoH* were both under regulatory control of σ^E, indicating that σ^E promotes its own transcription and the transcription of other sigma factors (Rhodius et al., 2006). By autoregulation, σ^E can create a multi-fold increase in gene product from its regulon. All of the genes found to be under the control of σ^E are related to making proteins for cellular structure.

3.4 σ^H – Response to heat shock

One of the genes under the transcriptional control of RpoE is another sigma factor, RpoH or σ^{32} (Rhodius et al., 2006). This sigma factor has been found to be involved in the transcription of genes that help *Salmonella* withstand high temperatures, potentially as a result of fever response within the host. Whereas σ^E appears to mediate the response to misfolded outer membrane

proteins, σ^H is involved with proteins within the cytoplasm that are misfolded (Bang et al., 2005). Concomitant with increased heat exposure, cell wall and membrane proteins begin to misfold and denature. As the concentration of σ^E increases in response to the misfolded proteins, σ^H also accumulates to respond to a sustained stressor. This is supported by the finding that *rpoH* expression is directly proportional to σ^E activity at temperatures above 42°C (Testerman et al., 2002), a temperature at which protein denaturing begins with the cell.

RpoH governs the transcription of genes such as those encoding proteases that allow for the removal of misfolded proteins within the cytoplasm. For example, an operon composed of *opdA* and *yhiQ* was found to be immediately proceeded by a consensus sequence for the RpoH promoter (Conlin & Miller, 2000). While the function of these two proteins has not been directly studied in the heat shock response, OpdA is metalloprotease oligopeptidase A that would be helpful in degrading misfolded proteins.

Some researchers have also hypothesized that σ^H is related to RNA thermometers, which are other regulatory means for activating and utilizing heat shock genes. RNA thermometers are areas of 5′-untranslated region that fold and complementary pair in such a way as to block the Shine-Dalgarno (SD) sequence of downstream genes (Waldminghaus et al., 2007). When heated to high enough temperatures, these areas unpair to allow the ribosome access to the SD sequence. A previously undescribed RNA thermometer was found within the 5′-UTR of the *agsA* gene in *S. enterica*. This gene is known to be involved in response to heat shock, and has a promoter region that is a consensus sequence for RpoH. Within the *agsA* mRNA appear to be RNA thermometer sequences (Waldminghaus et al., 2007).

In *E. coli*, the *rpoH* mRNA itself contains RNA thermometers. In this species, the cellular level of the RpoH is controlled by complementary base pairing in its mRNA. Unlike other RNA thermometers, the SD sequence is not blocked but the start codon is inaccessible to the ribosome and two halves of the ribosome-binding site pair at low temperatures (Waldminghaus et al., 2007). A similar mechanism is likely at play in *Salmonella*.

While responding to heat shock is vitally important for survival of the bacteria, the most important function of σ^H is to mediate σ^E regulation of σ^S through *hfq* gene expression. In *E. coli*, the promoter sequence found upstream of the *hfq* gene was found to be σ^H–dependent. The same promoter was found in *S.* Typhimurium (Bang et al., 2005). In conditions with scarce nutrients, σ^E appears to upregulate σ^S through the increase of σ^H (Bang et al., 2005).

The product of the *hfq* gene, HF-I, is important for translation of RpoS. This small protein is heat stable and binds to RNA to facilitate translation (Brown & Elliot, 1996) by associating with the ribosome (Brown & Elliot, 1997). Several possible mechanisms for the manner in which the protein encoded by *hfq* regulated σ^S translation have been suggested, including preventing the interaction of some sort of antisense mRNA or by being directly involved in the transcription of *rpoS* (Cunning & Elliot, 1999). Most evidence supports the assertion that the function of HF-I is as an RNA chaperone, after it was demonstrated to bring the mRNA and ribosome in correct association for translation (Sittka et al., 2007).

3.5 σ^S – Stationary phase growth, response to stress, and response to starvation

The role of this sigma factor, also called σ^{38}, is slightly more difficult to define than that of RpoE or RpoH. However, it is clear that the function of RpoS is essential. The conserved

sequence of *rpoS* across multiple species and within the same species found in different geographical areas speaks to its importance. When *rpoS* genes are characterized in clinical isolates, the mutations found are not clonal but rather novel, implying that there is some selection against mutants. Even when strains demonstrated different abilities to survive certain stresses like exposure to hydrogen peroxide, it did not appear to be related to different *rpoS* genes (Robbe-Saule et al., 2007).

The number and types of genes that seem to be under transcriptional control of σ^S have a variety of functions and respond to a wide variety of lifestyle requirements and threats to survival. The only known constant about the genes transcriptionally governed by RpoS is their dependence on growth phase (Ibanez-Ruiz et al., 2000). Previously, work has determined that during logarithmic growth, any activity from σ^S promoters is repressed by cyclic-AMP receptor activity (Fang et al., 1996). Stationary phase growth is characterized by a lack of cellular multiplication and decreasing cell density. The transition from exponential growth to stationary phase growth is the result of the concentration of a regulatory protein (Hirsch & Elliot, 2005). The concentration of Fis (factor for inversion stimulation), a DNA binding protein, is high during exponential growth and low in stationary phase. Fis binds to a region of DNA upstream of the promoter for *rpoS* and with decreasing concentration, allows the switch to stationary phase (Hirsch & Elliot, 2005).

A genome-wide search has been done for genes under the transcriptional control of RpoS. The project found that, like RpoE, the σ^S regulon includes promoters of various strengths. Despite the assumed similarities between the *E. coli* and *S.* Typhimurium genome, there were several genes within the *Salmonella* genome that were not homologous with any genes of *E. coli*. Several genes of unknown function were found under the control of σ^S, as was *ogt*, which encodes the enzyme O^6-methylguanine DNA methyltransferase (Ibanez-Ruiz et al., 2000). This enzyme is responsible for repairing DNA damaged by alkylation (Fang et al., 1992).

σ^S also seems to play a role in a wide variety of other functions that ensure the survival of the bacteria, such as protection from acid shock and nutrient depletion. Decreased pH unfolds the secondary structure stem and loops of the *rpoS* mRNA, allowing availability for translation (Audia & Foster, 2003). Constitutive degradation of the sigma factor coupled with no more being made results in the system reset after the acid threat has passed (Audia & Foster, 2003). RpoS also seems to be involved in survival of the bacteria in starvation conditions. σ^S has been found to act as both a positive regulator for *stiA* and *stiC* and a negative regulator for *stiB*. These three genes are part of the multiple-nutrient starvation-induced loci. σ^S was required for phosphate, carbon, and nitrogen starvation survival through induction of *stiA* and *stiC*. σ^S also acted as a negative regulator of *stiB* during phosphate and carbon starvation induced stationary growth (O'Neal et al., 1994).

3.6 σ^F – Flagellar formation and chemotaxis

Flagellar assembly was originally assumed to be under the control of σ^{70}, because it seemed essential to survival. However, examining promoters of known flagellar genes found no consensus sequences for σ^{70} (Helmann & Chamberlin, 1987). Instead, researchers found promoter sequences in *Salmonella* known to be used by alternative sigma factors in closely related species (Helmann & Chamberlin, 1987). σ^F, more commonly called FliA, or σ^{28}, has the most specific function of all the alternate sigma factors. FliA is involved in the transcription of genes related to the formation of flagella, specifically the formation of the

flagellar filament (Ohnishi et al., 1990). Operons of flagellar assembly are proceeded by one of three classes of promoters, class 1, 2, or 3 (Bonifield & Hughes, 2003, Karlinsey et al., 2000, Karlinsey et al., 2006) which allow for a temporal regulation of gene expression. From these operons, more than 50 genes are transcribed to allow complete flagellar assembly and function (Kutsukake et al., 1994).

There is only one class 1 operon which encodes the *flhD* and *flhC* genes (Karlinsey et al., 2000). Class 1 is the master operon, with FlhD and FlhC acting as a global regulator of flagellar assembly (Karlinsey et al., 2006). FlhD and FlhC form a heterotetrameric complex that is a positive transcriptional activator of class 2 promoters through σ^{70}, by interacting with the α subunit of the core enzyme (Bonifield & Hughes, 2003, Liu et al., 1995, Liu & Matsumura, 1994). Class 2 operons include genes for the assembly of the hook and basal body complex (HBB), σ^F, and FlgM (Bonifield & Hughes, 2003). The basal body, containing the motor, penetrates the cell membrane and includes the hook element on the extracellular side of the cell (Brown & Hughes, 1995). The filament protrudes from the hook into the extracellular matrix and turns to provide motility.

The third class of flagellar operons requires σ^{28} or FliA for transcription (Bonifield & Hughes, 2003). Proteins generated from these operons are for the flagellar filament, the generation of motor force, and chemotaxis (Karlinsey et al., 2006). FlgM, which is also transcribed from class 2 operons along with FliA, acts as an anti-sigma factor, keeping FliA inactive until the completion of the HBB. The C-terminal portion of FliA has a binding site for FlgM (Kutsukake et al., 1994). FlgM prevents RNA polymerase core enzyme from interacting with FliA to transcribe class 3 flagellar operons (Chadsey et al., 1998). The FlgM protein is able to assess the completion of the HBB because the protein itself is an exported substrate (Hughes et al., 1993). Decreasing concentrations of FlgM release FliA to interact with the RNA polymerase core enzyme and transcribe class 3 operons (Hughes et al., 1993). The relative concentration of FliA to FlgM determines the number of flagella that a single cell will have (Kutsukake & Iino, 1994). Additionally, the FlhD/FlhC complex may assist FliA in association with the RNA polymerase (Kutsukake & Iino, 1994). FlhD is involved in assessing nutrient state (Chilcott & Hughes, 2000), which may be requisite for bacterial motility.

The intracellular concentration of FliA and FlgM is governed by other regulatory mechanisms as well. The genes from both of these proteins can be transcribed from either class 2 or class 3 promoters (Wozniak et al., 2010). In this way, FliA can positively and negatively regulate its own intracellular concentration dependent upon the concentration of FlgM within the cell (Ikebe et al., 1999). Mutants lacking FlgM overproduce flagella via overexpression from class 3 operons (Yokoseki et al., 1996).

4. Changing partners

The presence of alternate sigma factors has been well studied, but how do the alternate sigma factors displace the housekeeping sigma or each other to govern gene transcription? Most of the answer points to concentration dependence; that is, the concentration of a particular sigma factor changes in response to different environmental conditions. For example, RpoE, as discussed above, is expressed constitutively but held inactive by various other proteins until an extracellular signal is received. This signal activates a series of proteolytic activities that gradually increases the intracellular concentration of RpoE. Once RpoE is released, it is free to interact with the core enzyme. RpoE is positively autoregulated and as genes are transcribed

from RpoE-response promoters, the intracellular concentration increases exponentially so that the intracellular concentration of RpoE can outcompete other sigma factors for binding access to the core enzyme. RpoE, in turn, allows for transcription of *rpoH*, which summarily mediates *rpoS* expression, increasing the intracellular level of all three alternative sigma factors. Fine tuning of these concentrations allows for precise control of gene expression. If a finite amount of RNA polymerase is available, increasing the presence of one sigma factor can repress expression of genes requiring a different sigma factor (Farewell et al., 1998).

Growth phase also appears to play a role in the intracellular concentration of certain sigma factors. During exponential growth, intracellular concentrations of σ^{70} remain relatively constant and σ^{S} is basically absent (Jishage & Ishihama, 1995). During stationary phase growth, the intracellular concentration of σ^{S} increases to nearly 30% of σ^{70} concentration (Jishage & Ishihama, 1995). Moreover, the concentration of the core enzyme decreases during stationary phase growth (Jishage & Ishihama, 1995), meaning that a 30% increase in concentration is more than a 30% increase in competitive advantage. RpoS activity is repressed by the products of *uspA* and *uspB*, which are both under the transcriptional control of σ^{70} (Farewell et al., 1998). During exponential growth, σ^{S} is highly unstable (Jishage & Ishihama, 1995). In stationary phase growth, σ^{S} is released and free to interact with RNA polymerase core enzyme. Researchers have hypothesized that there may be a σ^{70} anti-sigma factor under transcriptional control of σ^{S} or that a change in the cytoplasm may favor σ^{S} – mediated transcription (Farewell et al., 1998). Most genes expressed during exponential growth are not expressed during stationary phase growth, so σ^{70} proteins need to be rendered inactive (Jishage & Ishihama, 1995). Interestingly, the intracellular levels of σ^{S} reach those of σ^{70} during osmotic shock (Jishage & Ishihama, 1995), indicating that the change in concentration of a sigma factor can be a gradual or dramatic.

Environmental conditions can also play a role in the stability of the proteins, which can affect transcriptional efficiency. For example, RpoH, the heat shock sigma factor, is highly unstable at low temperatures; but, above 42°C intracellular concentrations will transiently increase (Jishage & Ishihama, 1995). Higher temperatures may provide increased efficiency of σ^{H} – mediated transcription or they may stabilize the protein itself so that it is able to interact with the core enzyme (Jishage & Ishihama, 1995).

5. *Salmonella* as pathogenic bacteria

In determining how alternate sigma factors are able to promote survival and spread of *Salmonella*, it is important to understand how *Salmonella* lives. *Salmonella* typically enters its host through the oral route. If sufficient numbers are ingested, some organisms will survive the low pH conditions of the stomach to reach the small intestine (Dougan et al., 2011). Sometimes the bacterial infection is halted here. For a systemic infection to occur, the bacteria must invade the gut epithelium (Hansen-Wester & Hensen, 2001). *Salmonella* preferentially invade epithelial cells in the distal ileum of the small intestine by adhering to and then injecting effector proteins into the host cell that facilitates bacterial entrance into membrane bound vesicles (Bueno et al., 2010). The small intestine provides an environment of near-neutral pH and high osmolarity, condusive to invasion not found in the large intestine (Lawhon et al., 2002).

Within the small intestines, *Salmonella* specifically invades Peyer's patches through M cells. Peyer's patches are specialized lymphoid tissues that are designed to sample intestinal

antigens and lead to immune responses (Slauch et al., 1997). *Salmonella* exclusively enter M cells found within the follicle-associated epithelium of Peyer's patches (Jones & Falkow, 1994). M cells are epithelial cells responsible for the uptake of luminal antigens (Slauch et al., 1997) and can engulf large particles, making them ideal for target by *Salmonella* (Jones & Falkow, 1994). When one bacterium makes entry into the host epithelial cell, it recruits other pathogens to its location (Francis et al., 1992).

6. Islands of pathogenicity

An estimated 5-10% of genes within the *Salmonella* genome can be considered virulence genes (Slauch et al., 1997). These genes have been found arranged in clusters within the *Salmonella* chromosome, the so called *Salmonella* Pathogenicity Islands (SPIs). It has been theorized that these gene clusters were acquired by horizontal transfer based on their higher G-C content compared with other parts of the *Salmonella* chromosome (Slauch et al., 1997) and because similar regions are not found in closely related commensal species such as *E. coli* (Galán, 1996). There are at least five known SPIs, but SPI-1 and SPI-2 seem to the most important in the initial phases of infection. Both SPI-1 and SPI-2 encode type III secretion systems (TTSS) (Shea et al., 1996). Additionally, genes within the SPIs encode effector proteins and regulatory proteins (Hansen-Wester & Hensen, 2001). These secretion systems allow the insertion of effector proteins into the extracellular environment and inside the host cell.

SPI-1 appears to contain genes involved in bacterial uptake by the host cell, while SPI-2 genes are involved in survival inside cells (Lara-Tejero & Galán, 2009). However, there is some evidence indicating that SPI-1 may also be important for bacterial life inside the vacuole and for their survival and replication intracellularly (Steele-Mortimer et al., 2002). Secreted proteins from genes transcribed from SPI-1 leads to actin cytoskeleton rearrangement of the host cell that facilitates bacterial entrance into membrane bound vesicles (Chen et al., 1996). Once inside the cell, a variety of functions can be hijacked to serve the bacteria's purpose, including cytoskeleton arrangement, vesicular trafficking, cell cycle progression, and programmed cell death (Lara-Tejero & Galán, 2009). These effector proteins activate GTP-binding proteins such as Cdc42, Rac-1, and Rho, which coordinate intracellular activities in the host cell (Chen et al., 1996). Effector proteins also down-regulate actin rearrangement (Fu & Galán, 1999) to reverse the actin rearrangement.

Transcription of all SPI-1 operons is activated by a regulatory loop beginning with HilA (Matsui et al., 2008). Through other regulator proteins like HilC, HilD, and InvF, expression of invasion genes is modulated with HilA as the central player (Lucas et al., 2000). Interestingly, the rising concentration of acetate in the distal intestine activates the expression of HilA, bypassing normal positive regulators (Lawhon et al., 2002).

While SPI-1 may play a role in the procession of the infection past the initial invasion of epithelial cells, SPI-2 is vital for the migration of the bacteria to other parts of the host (Löber et al., 2006). SPI-2 was the second pathogenicity island discovered and is required for virulence after the bacteria has entered into the epithelial cells (Shea et al., 1996). This claim is further supported by evidence that mutants without SPI-2 genes could enter Peyer's patches but were unable to spread to mesenteric lymph nodes (Cirillo et al., 1998). Not all members of the SPI-2 pathogenicity island are equally vital for the ability of the pathogen to

establish systemic infection. Mutants with various genes knocked out show a varying level of attenuation (Cirillo et al., 1998, Hensel et al., 1998). However, the genes within the SPI-2 are responsible for avoiding destruction by lysosomes within dendritic cells and macrophages (Tobar et al., 2006). Expression of SPI-2 genes seems to be induced by the slightly acidic conditions inside the initial vacuole formed when the bacteria are initially internalized by the host cell (Löber et al., 2006).

6.1 Regulation of *Salmonella pathogenicity islands* by sigma factors

Regulatory control of SPIs can be exerted by sigma factors without sigma factors being directly involved in the transcription of these genes. SPI-1 genes are typically transcribed using σ^{70}. σ^{H} mediates SPI-1 expression by regulating activators of SPI-1. Systems mediated by RpoH negatively regulate HilD post-translationally and HilA transcriptionally (Matsui et al., 2008). HilD is responsible for activating HilA transcription, and HilA in turn activates all the genes within SPI-1. σ^{H} directs the production of Lon protease which specifically degrades HilD (Matsui et al., 2008). By modulating the activation of σ^{H}, the bacterial cell can control SPI-1 expression, restricting expression to specific regions within the host cell (Matsui et al., 2008). The cell can repress invasion genes long enough to replicate, escape, and invade a new macrophage before cell death (Matsui et al., 2008).

Promoters for SPI-2 genes all have consensus sequences for σ^{70} (Osborne & Coombes, 2009). However, upstream of some SPI-2 genes seem to be consensus sequences for σ^{E} recognition (Osborne & Coombes, 2009). It is postulated that these σ^{E} binding sites may serve a couple of different purposes. The σ^{E} – recognized promoters may allow the bacteria to express TTSS in response to host factors that compromise bacterial cellular integrity (Osborne & Coombes, 2009). Alternatively, σ^{E} may fine-tune the expression of SPI-2 genes through σ^{70} (Osborne & Coombes, 2009) by preferentially overexpressing certain genes while all others are expressed at basal levels by σ^{70}.

Stationary phase *Salmonella* are unable to cause actin rearrangement in the host epithelial cell that is necessary for entry (Francis et al., 1992). Invasion factors are either not functional or not expressed in stationary phase bacteria (Francis et al., 1992). As growth phase has been demonstrated to change intracellular concentrations of different sigma factors and virulence genes do not appear to be under the transcriptional control of σ^{S}, it stands to reason that these bacteria would not be able to invade; invasion genes would be inactive since the activity of the necessary sigma factor is repressed.

6.2 Other genetic sources of virulence

Virulence genes may be found outside of *Salmonella* pathogenicity islands. These genes are similarly essential to survival and also are responsive to changes in sigma factor availability. While the genetic location of the Spv regulon varies among *Salmonella* species from chromosomal to plasmid-encoded, all species carry the regulon and it functions to increase intracellular growth in host cells once the bacteria have spread outside of the small intestine (Guiney et al., 1995). σ^{S} mutants are unable to efficiently express the Spv regulon. Expression of one of the members of the Spv regulon, *spvB*, decreased by 86% when σ^{S} was knocked out (Fang et al., 1992). The lethal dosage in mice for a strain without a functional *rpoS* gene was 1000 fold greater than the wild type (Fang et al., 1992).

The dependence of Spv regulon expression on growth phase also indicates a dependence on σ^S for transcription. However, it seems to be nutrient availability, not cell density, that is most important in mediating Spv regulon expression (Guiney et al., 1995). σ^S associated with RNA polymerase results in expression of genes that are essential to help the bacteria survive nutrient depleted conditions, such as those found in deeper tissues beyond the small intestine (Guiney et al., 1995).

σ^S increases expression of *spv* virulence genes by interacting with SpvR, a repressor protein for the virulence plasmid (Kowarz et al., 1994). Competition for RNA polymerase between σ^S and σ^{70} led to less efficient transcription of *spvR* from its promoter as σ^S has a greater affinity for RNA polymerase than σ^{70} but a lower affinity for the promoter for *spvR* (Kowarz et al., 1994). σ^S affinity for RNA polymerase is enhanced by its interaction with the Crl protein, giving it the ability to displace σ^{70} as the preferred promoter (Robbe-Saule et al., 2007). The presence of SpvR regulates its own transcription (Kowarz et al., 1994) so the lack of efficient transcription leads to decreasing cellular levels and derepression of *spv* plasmid virulence genes. σ^S ensures that enough SpvR is present to activate transcription from the *spvA* promoter, the first gene in the regulon (Guiney et al., 1995).

7. Sigma factors and surviving the best of times and the worst of times

While *Salmonella* Pathogenicity Islands allow the bacteria to invade host cells, the pathogen must then survive the hostile environment found inside. While differential gene expression from various sigma factors ensures the appropriate expression of SPIs to gain access to the intracellular milieu of the host, the use of alternate sigma factors also permits survival.

7.1 A sigma factor cascade for survival in phagocytic cells

Ferric Fang describes a cascade of transcriptional and translational events that involve sigma factors associating with the core enzyme to transcribe genes for each other and those necessary to respond to a variety of assaults in the intracellular environment (Fang, 2005). The first step in the cascade is activation of σ^E, which is constitutively expressed through σ^{70} promoters, but held inactive by a pair of negative regulators, RseA and RseB (De Las Peñas et al., 1997). RseA interacts with σ^E in such a way as to block the binding site for RNA polymerase (Muller et al., 2009). When an extracytoplasmic stress is perceived, σ^E is released by RseA and freed to bind to RNA polymerase. Interaction of σ^E with the core enzyme allows for transcription from other promoters. These promoters include those before the σ^E regulon of genes but also before the *rpoH* gene, which encodes the alternative sigma factor, σ^H. σ^H provides specificity for RNA polymerase to transcribe genes in the σ^H regulon, which respond to cytoplasmic stress. Additionally, σ^H allows transcription of *hfq*. The Hfq protein interacts with the *rpoS* mRNA to facilitate its translation. The σ^S then allows transcription of genes under its transcriptional control, which allow for a starvation response (Fang, 2005). This overall cascade allows for coordinated response by the pathogen. To ensure that sigma factors help transcribe genes needed to respond to stress only as long as it exists, there must be some mechanism of turnover (Fang, 2005). In this way, the use of an interconnected web of sigma factors allows *Salmonella* to gain access to various cell types and then survive to be able to spread to other areas of the host.

This cascade's vital importance to survival, in particular within macrophages, is illustrated by the increased levels of σ^S inside the macrophage following infection. Some aspect of

being inside a macrophage results in increased transcription of the *rpoS* gene. While levels of the housekeeping sigma σ^{70} decreased, levels of σ^S increased about 10-fold a few hours after infection (Khan et al., 1998). Conditions inside the macrophage induce the stress response and restrict nutrient availability, which induces the sigma cascade of gene expression to help the bacteria survive, although not necessarily to increase/induce virulence.

7.2 Sigma factors coordinate gene expression

Rarely is gene expression controlled in a strictly linear manner. That is, multiple sigma factors may work together to fine tune an expression of a group of genes to provide the bacteria with high probability of survival. The cascade of sigma factors used to allow survival inside phagocytic cells (described above) is just one example. There are many other instances of sigma factors working simultaneously.

One way to determine if one sigma factor plays a role in the efficient transcription by the other is to knock out one of them and see how the function of gene products mediated by the other are affected. In this way, investigators determined a relation between RpoE and FliA. Mutants without *rpoE* showed defective or limited mobility (Du et al., 2011). In these mutants, expression from class 1 flagellar promoters remained unaffected while some class 2 and most class 3 promoters showed decreased activity as compared to wild type (Du et al., 2011). It was concluded that RpoE may promote expression from class 3 promoters by mediating expression of FliA during osmotic stress, such as the hyperosmotic conditions found in the small intestine (Du et al., 2011).

RpoH and RpoN also appear to be related based on their ability to control the same genes as well as their dependence on one another. Expression of some heat shock operons appear to be under the control of RpoN in certain conditions, as expression from σ^H operons is down-regulated in mutants with an *rpoN* knockout (Studholme, 2002). In this way, RpoN may be responsible for fine tuning some gene expression during heat shock response. The expression of topoisomerases also appears to be governed by both σ^N and σ^H (Studholme, 2002), which may also indicate an interdependence of the activities of the two sigma factors.

Insufficient expression of one sigma factor can be compensated for by over-expression of other sigma factors. For example, researchers expected that because RpoS was vital to survival within macrophages, this sigma factor would be important for expressing virulence genes inside these phagocytic cells. However, within macrophages while RpoS only moderately increased following infection, RpoH and RpoE showed dramatic increases in intracellular concentration (Eriksson et al., 2003). While RpoS is typically associated with virulence inside phagocytic cells, it may be possible for other sigma factors to express other genes in response to a different environmental stimulus while still ultimately resulting in virulence. Research has also demonstrated that RpoN can compensate for insufficient RpoS in the formation of certain lipopolysaccharides (Bittner et al., 2004).

7.3 Survival outside of a host

While *Salmonella* is an important enteric pathogen to study because it infects many hosts and can be transmitted from species to species, it also is able to survive for long periods of time outside a host. Because of this characteristic, it has been an important target of sanitation processes to eliminate possible sources of transmission.

One mean of *Salmonella* transmission to human hosts is through food products, such as poultry. The same mechanisms of alternate sigma factor used to survive acid challenges in the mammalian gut are also utilized in surviving the fowl gastrointestinal tract and can lead to transmission of the pathogen (Dunkley et al., 2008).

Other studies specific to food handling procedures and alternate sigma factors have determined that RpoS, for example, is essential to *Salmonella*'s ability to withstand normal sanitation procedures common in the food service industry and that early induction of RpoS can cause the cells to enter stationary growth phase prematurely, negating the protective nature of stationary growth on the pathogen's ability to survive (Komitopoulou et al., 2004). Other studies have demonstrated that certain food handling processes, such as washing in various antimicrobial agents, can induce RpoS to protect the bacteria from destruction (Dodd & Aldsworth, 2002). Significant drops in temperature have also been found to activate transcription from σ^S dependent promoters rather than from the σ^{70} promoters from which genes are normally transcribed (Rajkumari & Gowrishankar, 2001).

Multiple alternate sigma factors contribute to survival through food processing. For example, σ^S and σ^E were both found to be important in surviving refrigeration and changes in osmotic pressure. Depending on the nature of the stress, either σ^S or σ^E may be more important and their relative concentrations dictate the response (McMeechan et al., 2007).

8. Transcriptional and translational regulation of sigma factors

Because sigma factors are capable of effecting dramatic changes in cellular protein composition and energy use, their actions must be closely guarded to ensure that the pathogen is responding to the stress without exhausting cellular resources.

8.1 Regulation of sigma factors

Some alternate sigma factors are constitutively expressed but held inactive until they are needed by regulatory proteins that change conformation or leave the cell in response to a particular signal. RpoE is held inactive until an extracellular signal of extracytoplasmic stress is received and FliA is held inactive by FlgM until the FlgM is exported out of the cell by the completed hook and basal body structure. Some regulation of sigma factors is accomplished by the optimal conditions under which they can influence gene expression. *rpoH* cannot be translated below a certain temperature because at lower temperatures the mRNA folds back on itself blocking the start codon. And RpoS shows increased efficiency during stationary phase growth and is almost nonexistent during exponential growth.

Because much of the efficiency of sigma factors to influence transcription is itself influenced by their relative concentrations within the cells, many mechanisms to regulate them change the available concentration of these proteins. Different proteases target specific sigma factors and depending on the relative concentration of these proteases, the relative availability of the sigma factors can be adjusted.

RpoS is needed to transcribe the most genes and is therefore the most highly regulated. Several novel pathways of regulation have been discovered. DksA is required for efficient translation of *rpoS* but not as an RNA chaperone (Webb et al., 1999). Another protein, RstA, decreases the expression of RpoS controlled genes and appears to decrease cellular levels of

RpoS independently of proteolytic activity (Cabeza et al., 2007). Translation of the *rpoS* mRNA is elevated in the presence of appropriate carbon sources, indicating a growth rate dependent control of sigma factor availability (Cunning & Elliot, 1999). In response to increased glucose levels, StpA prevents overactivation of σ^S indirectly enhancing its turnover (Lucchini et al., 2009). Some small mRNAs such as DsrA and RprA, are highly conserved as are their antisense elements within the *rpoS* mRNA, but they only have small effects on RpoS availability (Jones et al., 2006). DsrA interaction with *rpoS* mRNA disrupts the stem and loop base pairing of *rpoS* mRNA to allow high levels of translation (Majdalani et al., 2001). The same study discovered another small RNA, RprA, that interacts in a similar way to positively regulate RpoS translation (Majdalani et al., 2001).

8.2 Sigma factors and other regulatory mechanisms

Differential gene expression through alternate sigma factors is far from the only regulatory mechanism found in *Salmonella*. When these other regulatory systems respond to environmental stimuli, alternate sigma factors influence gene expression related to these systems as well. Two important regulators that intersect differential gene expression with sigma factors are the PhoP/PhoQ regulatory system and the Fis global regulator.

The PhoP/PhoQ regulatory system influences the expression of many genes and is functionally a sensor of extracellular magnesium concentration. It has been hypothesized to have evolved differently in closely related species like *E. coli* and *Salmonella* as a result of different lifestyles (Monsieurs et al., 2005). The relation between the PhoP/PhoQ regulatory system and σ^S appears to be essential. Even in cells with functional copies of *rpoS*, mutants lacking PhoP cannot form functional phagosomes within phagocytic cells (Alpuche-Aranda et al., 1994). Mutants with a double knockout of the RpoS and PhoP/PhoQ show decreased virulence and decreased invasion of host cells (Lee et al., 2007). It has even been suggested that because of their inability to cause lasting infections, these double knockouts should be used to make a *Salmonella* vaccine (Lee et al., 2007).

PhoP controls the level of available RpoS by controlling proteins, which enable its degradation by ClpXP. PhoP acts as a transcriptional activator for *iraP*, which encodes a protein that interacts with RssB. RssB facilitates ClpXP degradation of σ^S (Tu et al., 2006). By blocking RssB activity, the level of σ^S accumulates during PhoP/PhoQ activation, which includes low levels of magnesium as found inside macrophages. This is very different than the type of regulation seen in the commensal *E. coli* (Tu et al., 2006), indicating that while there is some similarity in the genes expressed between the two, the regulation of the alternative sigma pathways is not the same. Interestingly, RpoE seems to be involved in the regulation of PhoP/PhoQ activity through Hfq, the same RNA chaperone through which it mediates RpoS expression (Coornaert et al., 2010).

Fis (factor for inversion stimulation) is a global transcription regulator and facilitates site-specific DNA recombination (Mallik et al., 2004). The intracellular concentrations of Fis are high during exponential growth and low in late exponential and stationary phase growth (Walker et al., 2004). The *fis* promoter itself is of some interest as to how these concentration differences are maintained. The σ^{70} dependent and growth-phase dependent regulation from this promoter is achieved through a weak -35 sequence, a second RNA polymerase binding site, and the relative concentration of nucleotides within the cell (Walker et al., 1999). The *fis*

promoter is somewhat unique among σ^{70} – dependent promoters in that transcription begins with a cysteine (Walker et al., 2004). This residue is normally a poor initiator of transcription and as a result the RNA polymerase holoenzyme binds very weakly with the *fis* promoter (Walker et al., 2004). When cellular concentrations of cysteine are low, there is very little transcription from the promoter but as CTPs increase in the cell, so does gene expression from the *fis* promoter (Walker et al., 2004).

As expected from the pattern of Fis concentration in the cell, there is a negative relationship between the intracellular level of RpoS and Fis during stationary phase growth (Cróinín & Dorman, 2007). Fis in fact is able to mediate expression from σ^S – dependent genes by binding to a Fis-specific site upstream of σ^S promoter regions and blocking RpoS activity during exponential growth (Hirsch & Elliot, 2005).

Fis, as its name suggests, is also essential for the ability of *Salmonella* to switch flagellar types. There are two types of flagellar filaments, FljB and FliC, which are both transcribed from class 3 promoters. Flagellar switching is achieved by inversion of a promoter region. When expression occurs from this promoter, a type B filament is produced and a repressor of type C is created. When the inversion occurs, the repressor of type C is not produced and type C filaments are made (Aldridge et al., 2006). Hin (for H invertase) and Fis are both required for proper inversion (Bruist et al., 1987). Hin seems to mediate the inversion while Fis ensures the appropriate alignment of the inverted DNA (Bruist et al., 1987).

In having two different types of filaments available for use, *Salmonella* is able to evade the host immune system. FliC is a well-studied target of the immune system (Cummings et al., 2005). As bacteria migrate through the small intestine and into the rest of the host, FliC expression is suppressed or switched for FljB expression to avoid detection by T cells (Cummings et al., 2005). Once past the initial site of infection, T cells are no longer able to recognize the pathogen (Cummings et al., 2005).

Finally, the relatedness of alternate sigma factors and pathogenicity can ensure that certain genes are not expressed at the wrong time. The gene *hilA* which is responsible for the regulation of SPI-1 genes is found in the same operon as FliA, the alternate sigma factor for flagellar filament assembly (Lucas et al., 2000). This proximity within the genome allows for the simultaneous control of both mobility and invasion properties, and ensures the likely co-inheritance of the regulatory elements.

9. Conclusion

Differential gene expression through the use of alternate sigma factors is one of numerous regulatory methods available to *Salmonella* to avoid destruction by its host's immune system or sanitation processes and to thrive in a variety of environments. Control through sigma factors intersects control exerted by other regulatory pathways to ensure a highly controllable pattern of gene expression. The full capacity of *Salmonella* to change rapidly and accurately to respond to environmental conditions is still not well understood. Genes that are under the most types of regulatory control are typically the most important in virulence (McDermott et al., 2011) and it is clear that not only are sigma factors highly controlled themselves at the level of transcription and translation, but they are interconnected in a complex web.

From a medical standpoint, rendering *Salmonella* essentially commensal by knocking out various genes for sigma factors may be an area of interest in creating vaccines. *Salmonella*

mutants with one or more nonfunctional copies of genes for alternate sigma factors show significantly attenuated growth across hosts and especially in macrophages, which seems to be the most essential characteristic of *Salmonella's* ability to evade the host immune system. Understanding how sigma factors protect the integrity of the bacteria and testing the limits of this protection may provide insight into the development of new sanitation processes that eliminate more of the bacteria and prevent spread.

10. Acknowledgment

11. References

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