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Review

Resistance and survival strategies of *Salmonella enterica* to environmental stresses

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ABSTRACT

Serovars of *Salmonella enterica* are frequent agents of foodborne disease worldwide. They are capable of growing and surviving in numerous natural, commercial and host environs where they must be able to sense and respond appropriately to the variety of environmental cues encountered. Many of these environments produce stresses to the cell in the form of nutrient limitation/starvation, acid/base, high/low temperatures, high/low osmolarity, desiccation, and exposure to antimicrobial peptides, bile salts and oxidizing agents. The response generated to a particular stress can provide a stress-specific resistance or a more general cross-resistance to a variety of deleterious conditions. Stress responses in *Salmonella* are controlled by an assortment of regulators – such as alternative sigma factors (*e.g.*, σ^S, σ^E, and σ^H), phospho-relay-based two component systems (*e.g.*, BaeRS, CpxRA, OmpR-EnvZ, PhoPQ, PmrAB (BasRS), and RcsBCD) and transcriptional regulators (*e.g.*, SoxS/SoxR, OxyR, Fur, RamA, RamR, MarA and MarR) – in response to environmental signal(s). Ultimately, these regulators result in the increased and/or decreased expression of sets of genes both unique to the specific stress response and overlapping with other stress responses. These stress responses generate a resistance that allows these enteropathogens to survive and persist in a variety of natural (*e.g.*, soil and water systems), food processing and handling, and host environments. Thus, these stress responses and survival strategies can have a profound impact on the epidemiology and pathogenesis of these medically and economically important bacteria.

Contents

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1. Introduction

Optimal growth conditions of plentiful nutrients and perfect growth temperature, pH, oxygen levels and solute levels are achieved only by microorganisms that are grown in the research laboratory. Under these conditions, these microbes will grow at their maximum growth rate or generation time. However, variation in any of these parameters, much above or below these optimums, will perturb the maximum growth rate and, therefore, represents a stress to the microbial cell. Because growth and survival under stressful conditions is the norm, the responses and survival of bacteria and other microbes during exposure to environmental stresses has become an important area of study in microbiology.

In order to survive sudden, potentially lethal, changes in the environments that they encounter, bacteria must be able to sense and respond rapidly and appropriately to a vast array of stresses. This is particularly vital for foodborne microbial pathogens that can encounter potentially life-threatening conditions in virtually every environment they may find themselves including: natural (*e.g.*, soil, water systems), commercial (*e.g.*, slaughter houses, food processing plants) and host (*e.g.*, animals, humans) settings (Winfield & Groisman, 2003). Responses to these conditions not only impact growth and survival but can also influence virulence and resistance to multiple antimicrobics (Altier, 2005; Clements, Ericksson, Tezcan-Merdol, Hinton, & Rhen, 2001; Dodd, Richards, & Aldsworth, 2007; Grant et al., 2009; Kenyon & Spector, 2011; McMahon, McDowell, & Blair, 2007; McMahon, Xu, Moore, Blair, & McDowell, 2007; McMeechan et al., 2007; Rowley, Spector, Kormanec, & Roberts, 2006). Few microorganisms are as capable of coping with the range of stresses present in natural, commercial and host microenvironments as *Salmonella enterica* serovars (Cabello, Hormaeche, Mastroeni, & Bonina, 1993; D' Aoust, Maurer, & Bailey, 2001; Kenyon & Spector, 2011; Rychlik & Barrow, 2005; Stocker & Makela, 1986).

Serovars of *S. enterica* are some of the most common agents of foodborne illness in the world. Members of this genus are capable of colonizing and causing disease in both animals (*e.g.*, poultry, cattle, swine, rodents) and humans. Some serovars like *S.* Typhimurium (the best studied) and *S.* Enteriditis infect a broad range of animal and human hosts, while others, such as *S.* Typhi are restricted to specific hosts (Stevens, Humphrey, & Maskell, 2009). As a group, *Salmonella* are skilled at adapting to, growing and/or surviving in a diverse range of stressful environments including: extracellular pHs down to 3.99 and up to 9.5, salt concentrations up to 4% w v^{-1} NaCl and temperatures as high as 54 °C or low as 2 °C (D' Aoust et al., 2001). Thus, these stresses can have a significant effect on the survival of salmonellae during food processing, preparation and storage as well as its passage through the host organism.

Salmonella serovars, upon evacuating from the host, generally, enter an aquatic environment (*e.g.*, municipal water/sewage systems or field run-offs) that can initially be relatively rich in nutrients but can rapidly become nutrient-depleted as a result of dilution. This rapid departure from more optimal conditions can also result in the bacteria encountering temperature downshifts, declining osmolarity and variability in pH. Over time, the bacteria may enter a dormancy or viable-but-nonculturable (VBNC) state, in which they can subsist for prolonged periods of time (Foster & Spector, 1995; Roszak, Grimes, & Colwell, 1984; Turpin, Maycroft, Rowlands, & Wellington, 1993). Additionally, the bacteria can undergo predation by certain protozoa/amoeba in which they can persist (Barker & Brown, 1994). Both animals and humans will typically become infected with salmonellae following ingestion of contaminated food or water. For humans, important sources of *Salmonella* serovars can include: contaminated or infected beef, pork, eggs, poultry, fruits, vegetables or derivatives/by-products of these foods, *e.g.*, peanut butter.

Salmonella serovars, being foodborne pathogens, must resist or evade multiple levels of defenses during pathogenesis within a host. Upon entering a host via ingestion of contaminated food or water, it first encounters the acidic pH of the stomach. Survivors entering the intestines must deal with reduced oxygen, bile salts, antimicrobial peptides, weak acids (metabolic products of resident microbial flora), increased osmolarity, and competition with resident microorganisms for nutrients and space (Rychlik & Barrow, 2005). Serovars able to adapt and survive these conditions typically colonize and invade the host intestinal mucosa through M cells by promoting their own endocytosis, winding up in a vacuole lacking in nutrients. Subsequent release into the intestinal submucosa is followed by phagocytosis by resident

macrophages allowing the bacteria to escape host humoral defenses. Within the macrophage, the salmonellae reside within phagosomes or possibly phagolysosomes, which present many dangers to the invading bacteria (*e.g.*, acidification, nutrient limitation, reactive oxygen and nitrogen species generation, and exposure to various antimicrobial peptides (*e.g.*, defensins). Spread of *Salmonella* serovars, beyond the intestines or associated lymphoid tissue is dependent on the host and/or the host's immunocompetence (Clements et al., 2001; Finlay & Falkow, 1989).

This review attempts to summarize the responses and strategies employed by *Salmonella* serovars (primarily *S.* Typhimurium which is the most extensively studied serovar) to prevail over the vast array of stresses encountered during their life cycles. Table 1 summarizes the roles of known regulatory networks – discussed in this review – in the responses that *Salmonella* serovars elicit to various stresses.

2. Stress responses and survival strategies

2.1. Starvation stress

A common stress encountered by bacteria, in general, is starvation for an essential nutrient, *e.g.*, carbon (C), phosphate (P), and/or nitrogen (N) sources (Harder & Dijkhuizen, 1983; Koch, 1971; Spector, 1998). As a result of their life cycle, salmonellae often suffer periods of nutrient starvation as they voyage through different natural, commercial, and host microenvironments they encounter (Abshire & Neidhardt, 1993; Dodd et al., 2007; Fang et al., 1992; Grant et al., 2009; Humphreys, Stevenson, Bacon, Weinhardt, & Roberts, 1999; Koch, 1971; Roszak et al., 1984; Rychlik & Barrow, 2005; Spector, 1998; Testerman et al., 2002; Turpin et al., 1993; Winfield & Groisman, 2003). Unlike endospore-forming bacteria, *Salmonella* and other enterobacteria depend upon different types of "programmed" physiologic responses for survival during periods of nutrient starvation, that are functionally analogous to sporulation but do not technically result in a structurally distinct "differentiated" cell form (*i.e.*, an endospore).

When *S.* Typhimurium is starved for a carbon-energy (C) source it undergoes global genetic and physiologic changes referred to as the starvation-stress response (SSR) (Kenyon, Sayers, Humphreys, Roberts, & Spector, 2002; Spector, 1990; Spector, 1998; Spector, Aliabadi, Gonzalez, & Foster, 1986; Spector & Foster, 1993; Spector, Park, Tirgari, Gonzalez, & Foster, 1988). Although the SSR and the stationary-phase (or general resistance) response can overlap in many ways there are important differences. For example, during the SSR the bacteria are specifically being starved for a C source, *e.g.*, glucose; while the limiting/stress condition(s) that generates stationary-phase cells is not clearly defined and typically involve multiple simultaneous stresses (Spector, 1998; Spector et al., 1986, 1988; Spector & Foster, 1993). In addition, cells referred to as stationary-phase cells are typically grown overnight in a rich medium (*e.g.*, LB medium) to relatively high cell densities, whereas C-starved cultures are typically grown to and starved at ~100-fold lower cell densities (Fang, Krause, Roudier, Fierer, & Guiney, 1991; Foster, 1991; Foster & Hall, 1990; Humphreys et al., 2003, 1999; McLeod & Spector, 1996; Testerman et al., 2002). These differences have significant effects on overall cell metabolism and survival. Interestingly, phosphate (P) or nitrogen (N) source starvation will also trigger a starvation-stress response in *S.* Typhimurium, but these are not as effective or broad as the response elicited by C-starvation (Foster & Spector, 1986; McLeod & Spector, 1996; Spector et al., 1988; M. Spector, unpublished data).

Carbon-starved *S.* Typhimurium, and other enterobacteria, cells are structurally and metabolically very different from actively growing, non-starved cells (Huisman, Seigele, Zambrano, & Kolter, 1996; Spector, 1998). The SSR can be thought of as a series of (overlapping) phases analogous to but not as defined as the stages of sporulation (Spector, 1998; Spector & Foster, 1993; Stephenson & Lewis, 2005). During the initial hours of C-starvation the primary goal of the cell is to avoid starvation by up-regulating alternative C source utilization and transport systems. As C-starvation continues, avoidance yields to more extensive metabolic changes that yield a smaller, hardier, and more physiologically efficient cell, compared with actively growing cells. The overall result of eliciting the SSR is the global "reprogramming" of cellular metabolism. This includes the production of: (i) new or higher affinity substrate transport and utilization systems (in the absence of substrate) for the scavenging of nutrients from the environment if they become available, (ii) enzymes for the "cannibalization" or turnover of unneeded cellular components, *e.g.*, RNA and proteins from ribosomes as well as lipids and peptidoglycan from the cell envelope, (iii) enzymes for the more efficient and complete metabolism of (unusual) C-sources, (iv) proteins that cause chromosome condensation protecting it from damage, (v) enzymes that modify

Table 1
Regulatory proteins/systems playing roles in stress resistance in *Salmonella enterica* serovars.

Regulator(s)	Stress[a]										
	C-starvation	Acid	Oxidative	Heat	Envelope	AP	Bile	Multi-drug	Osmotic	Dessication	Iron
σ^H			✓	✓							
σ^S	✓[b]	✓	✓	✓					✓	✓	
σ^E	✓	✓	✓	✓	✓	✓					
AdiY		✓									
BaeRS					✓		✓	✓			
CpxRA				✓	✓	✓					
cAMP-CRP	✓										
CsgD										✓	
DksA		✓	✓								
Fur		✓	✓								✓
LexA			✓								
MarA							✓	✓			
OmpR-EnvZ		✓							✓		
PhoPQ		✓				✓	✓(?)				
PmrAB						✓					✓
OxyR			✓				✓				
RamRA							✓	✓			
RcsBCD					✓	✓					
RecA		✓	✓				✓				
SoxRS			✓				✓	✓			
SlyA			✓			✓					

[a] See text for further explanation of the role of the regulator in the response to the specified stress.
[b] ✓ Indicates that the regulator plays a role in resistance to indicated stress; a blank indicates that the regulator either has no role or has not been reported to have a role in *Salmonella*.

inner membrane, peptidoglycan and outer membrane components [*e.g.*, the types and amounts of fatty acids in membrane lipids or the lipopolysaccharide (LPS) of the outer membrane], and (vi) enzymes to prevent or repair cellular damage as a result of environmental stresses (Almirón, Link, Furlong, & Kolter, 1992; Dougherty & Pucci, 1994; Druilhet & Sobek, 1984; El-Khani & Stretton, 1981; Hengge-Aronis, 1999; Huisman et al., 1996; Humphreys et al., 2003; Kenyon et al., 2007; Kenyon, Humphreys, Roberts, & Spector, 2010; Kenyon, Thomas, Johnson, Pallen, & Spector, 2005; Matin, 2009; Skovierova et al., 2006; Spector, 1998; Spector & Cubitt, 1992; Spector, DiRusso, et al., 1999; Spector, Garcia del Portillo, et al., 1999). Microarray analyses comparing C-starved *S.* Typhimurium to growing/non-starved cells indicates that there are approximately 160, 500 and 1300 open reading frames (ORFs) exhibiting at least 10-, 5- and 2-fold induction, respectively, in response to C-starvation (M. Antonio, S. Lucchini, R. Chaudhuri, A. Thompson, J. Hinton, M. Spector & M. Pallen, unpublished data). The majority (~70%) of the genes exhibiting induction are either known or proposed to function somehow in carbon-, nitrogen- or phosphate-source utilization and transport, energy generation and respiration, regulation and/or stress protection. About 20% of the up-regulated genes have unknown or putative functions (several are unique to *Salmonella* serovars), and about 5% represent known or proposed virulence genes (including several fimbriae and pathogenicity island gene clusters).

The function of the SSR in *S.* Typhimurium, and other enterobacteria, is to generate resistance to the damaging effects of long-term C-starvation (starvation-survival) and a variety of other environmental stresses [*a.k.a.*, C-starvation-inducible (CSI) cross-resistance or general resistance]. Several genes have been identified as members of the SSR stimulon in *S.* Typhimurium using a variety of different approaches (Hengge-Aronis, 1999; Huisman et al., 1996; Ibanez-Ruiz, Robbe-Saule, Hermant, Labrude, & Norel, 2000; Kenyon et al., 2002; Kenyon et al., 2007; Matin, 2009; O'Neal et al., 1994; Seymour, Mishra, Khan, & Spector, 1996; Spector et al., 1986; Spector et al., 1988; Spector & Cubitt, 1992; Spector, DiRusso, et al., 1999; Spector & Foster, 1993; Spector, Garcia del Portillo, et al., 1999).

2.1.1. Global regulation of the SSR — overview

In *S.* Typhimurium, the SSR is regulated at the global level by at least two signal molecules; cyclic 3′,5′-adenosine monophosphate (cAMP) with its receptor protein (CRP) and guanosine 5′-(tri or) diphosphate-3′-diphosphate [(p)ppGpp]; and, at least two sigma factors including σ^S and σ^E (Fang, Chen, Guiney, & Xu, 1996; Ibanez-Ruiz et al., 2000; Kenyon et al., 2002; Matin, 2009; McMeechan et al., 2007; O'Neal et al., 1994; Rowley et al., 2006; Skovierova et al., 2006; Spector, 1990; Spector, DiRusso, et al., 1999; Spector, Garcia del Portillo, et al., 1999; Testerman et al., 2002).

The intracellular levels of cAMP and (p)ppGpp both increase early during the SSR. The level of cAMP increases by activating and/or up-regulating the expression of adenylate cyclase (*cyaA* gene), as well as CRP (*crp* gene), in response to decreased glucose uptake as extracellular glucose levels drop (Görke & Stülke, 2008). Increased formation of the cAMP-CRP complex then goes on to activate or repress the expression of various genes in response to declining glucose levels. Many of these are genes involved in the transport and utilization of alternative carbon-energy sources, as discussed below. Interestingly, cAMP-CRP is found to repress several SSR loci during exponential growth conditions (*e.g.*, high glucose levels); this repression is overcome in C-starved cells even though cAMP-CRP levels are rising (Spector, 1990, 1998; Spector et al., 1988).

The levels of (p)ppGpp can rapidly rise by increasing the activity of (p)ppGpp synthetase I (*relA* gene) or the bi-functional (p)ppGpp synthetase II/guanosine-3′,5′-bis pyrophosphate 3′-pyrophosphohydrolase (*spoT* gene), respectively (Potrykus & Cashel, 2008). This primarily results from effects on protein synthesis resulting from declining carbon-energy source availability, which slow or block ribosome movement along the mRNA. The role of (p)ppGpp in bacterial stress physiology has become more clear over the last few years. (p)ppGpp has been shown to bind to RNA polymerase (RNAP) altering the transcription from several promoters, *e.g.*, ribosomal RNA genes, during the stringent response. In addition, a small protein DksA (*DnaK* suppressor) also plays a role in the stringent response in *E. coli* (Sharma & Chatterji, 2010). One of the roles that (p)ppGpp and DksA (along with the anti-σ^{70} factor Rsd and a 6S RNA) are proposed to have is to modulate σ^{70} activity which ultimately leads to increased RNAP binding with alternative sigma factors, *e.g.* σ^S and σ^E, in starved/stationary phase cells (Sharma & Chatterji, 2010). Although, this has not been reported for *Salmonella*, a similar scenario is likely. Indeed, both (p)ppGpp and DksA are reported to regulate C-starvation/stationary-phase gene expression (Henard, Bourret, Song, & Vázquez-Torres, 2010; Spector, 1998). Furthermore, Webb, Moreno, Wilmes-Riesenberg, Curtiss, and Foster (1999) reported that DksA plays a role in regulating σ^S levels during acid stress by controlling translation of the *rpoS* mRNA.

The levels and activity of σ^S increase by a very complex combination of transcriptional, translational and proteolytic controls that depend on the conditions encountered (Hengge-Aronis, 1999, 2002; Hengge, 2008; Navarro Llorens, Tormo, & Martínez-García, 2010; Fig. 1). There are some key differences between *E. coli* and *Salmonella* in terms of the control of *rpoS* expression. For example, the regulatory small RNA (sRNA) molecules DsrA and RprA have diminished roles in the translational regulation of *rpoS* in *Salmonella* and the H-NS paralog StpA indirectly promotes σ^S proteolysis by repressing the anti-adaptor protein *iraM* (*rssC*) gene in mid- and late-exponential phase cells, both in apparent contrast to *E. coli* (Jones, Goodwill, & Thomas Elliott, 2006; Lucchini, McDermott, Thompson, & Hinton, 2009).

The levels and activity of σ^E are controlled by a sequential system of membrane (*i.e.*, regulated intra-membrane proteolysis or RIP) and cytoplasmic (*i.e.*, ClpXP proteasome) proteolytic events as well as autoregulation of *rpoE-rseABC* operon transcription (Rowley et al., 2006; Ades, 2008; Fig. 2). A proposed mechanism for activation of σ^E during C-starvation likely involves the global increase in carbohydrate uptake systems along with the remodeling of the bacterial envelope. In this model, the periplasm is "flooded" with proteins, which overwhelms the levels of periplasmic proteases/chaperones/peptidyl-prolyl isomerases (PPIases) and increases the levels of misfolded proteins. This is supported by the finding that shifts from glucose to certain alternative C-sources result in σ^E activation. Of the several C-sources tested, only those whose utilization involved a periplasmic and/or outer membrane protein component (*e.g.*, maltose, citrate, and succinate) resulted in sustained σ^E activation (Kenyon et al., 2005). Furthermore, overexpression of LamB from a plasmid resulted in σ^E activation even though it lacks the Y-x-F peptide shown to activate the DegS protease triggering the RIP cascade (Kenyon et al., 2005). Furthermore, LamB overexpression appears to promote σ^E activation by indirectly increasing OMP (*e.g.*, OmpC) misfolding (W.J. Kenyon, A. Frank, K. Raveendran, & M.P. Spector, unpublished data).

The relative levels of σ^S rise early during the SSR peaking at around 5 h of C-starvation before declining to lower steady-state levels in 24–48 h C-starved cells. In comparison, σ^E levels peak at around 48–72 h of C-starvation (Fig. 3). The accumulation of σ^E and σ^S in the SSR correlates with increased expression of σ^E- and σ^S-dependent promoter activity (Kenyon et al., 2002; W.J. Kenyon & M.P. Spector, unpublished data). *S.* Typhimurium strains lacking the ability to make ppGpp (*relA spoT* double mutants), σ^S (*rpoS* mutants) or σ^E (*rpoE* mutants) are all unable to generate a maximal SSR (Kenyon et al., 2002; O'Neal et al., 1994; Spector, 1990; Spector & Cubitt, 1992; Spector et al., 1988; M.P. Spector, unpublished data). Interestingly, strains lacking CRP (*crp* mutants) generally exhibit increased stress resistance during exponential-phase growth, perhaps a result of the fact that cAMP-CRP functions as a exponential-phase repressor for many SSR loci (M.P. Spector, unpublished data).

2.1.2. Scavenging and utilization of alternative carbon-energy sources

One category of genes identified includes known or putative transport and/or catabolic utilization systems for alternative or unusual C-sources.

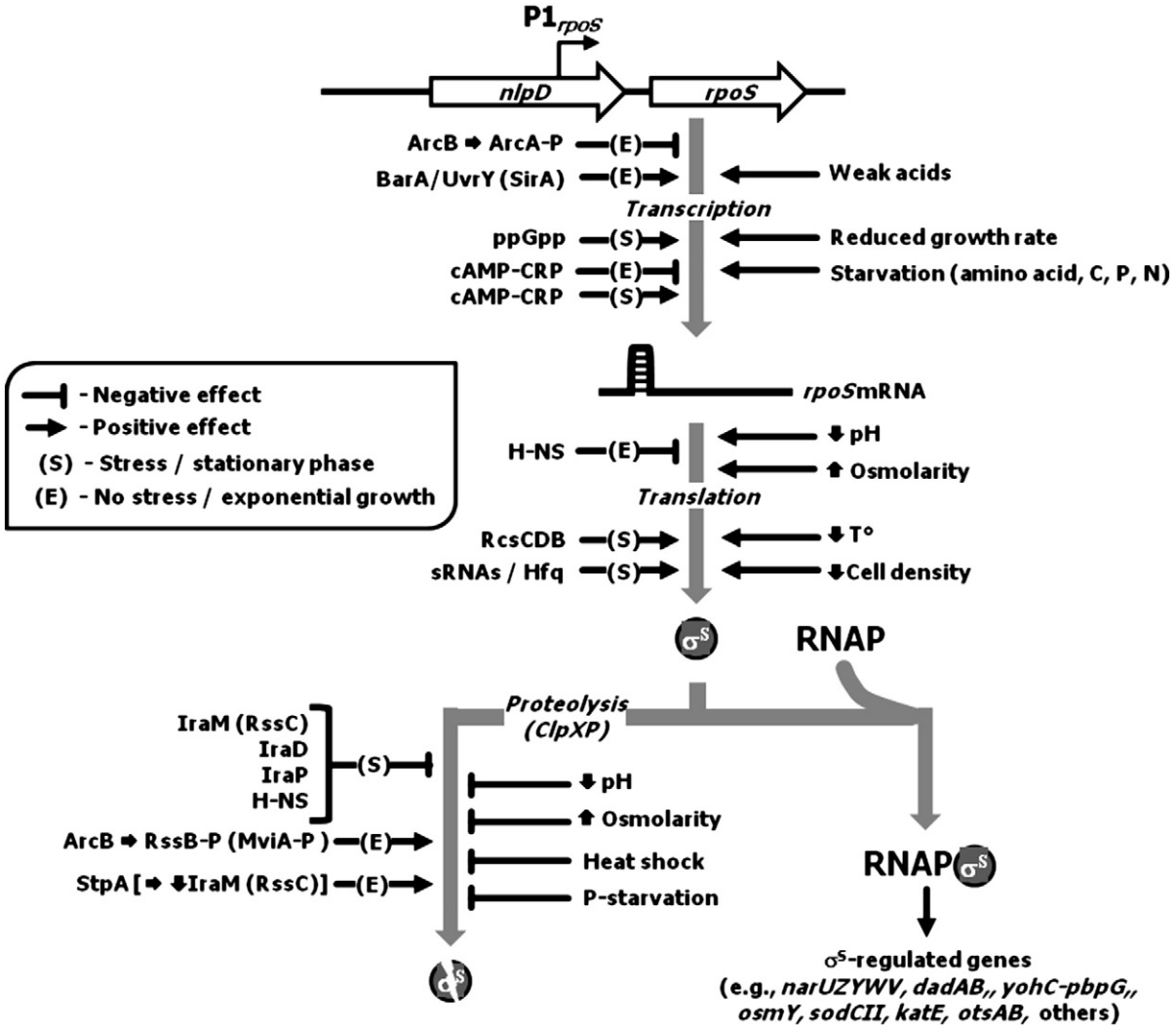

Fig. 1. Overview of the regulation of *rpoS* gene transcription, *rpoS* mRNA translation and σ^S proteolysis/stability by various regulatory factors and environmental stress conditions in *Escherichia coli* and *Salmonella* (Hengge, 2008; Navarro Llorens et al., 2010). Regulatory factors involved in *rpoS* control are indicated on the left side of the flow diagram while environmental stress conditions regulating *rpoS* expression are indicated on the right side of the diagram. See the corresponding text for additional explanation. The genes listed are examples of regulon members; see the corresponding text for additional genes and explanations. ⊢, indicates a negative effect on targeted step; ➵, indicates a positive effect on targeted step.

Among these are several known and uncharacterized Enzyme I- and Enzyme II-like components of phosphoenolpyruvate (PEP): carbohydrate phosphotransferase systems (PTS), MFS-family transporters, and periplasmic-binding protein/ABC transporter systems for an array of generally uncommon sugars (*e.g.*, fuculose, xylulose), sugar alcohols (*e.g.*, galactitol, glucitol, sorbitol, xylitol), and amino-sugars (*e.g.*, *N*-acetyl-glucosamine, *N*-acetyl-galactosamine, *N*-acetyl-mannosamine) and other C-compounds (*e.g.*, glucarate, galactarate). In addition, known or putative genes for the metabolism of alternative C-sources – such as ethanolamine (*eut* genes), propanediol (*pdu* genes), and aldehydes including glycolaldehyde and lactaldehyde (*e.g.*, *aldB*, aldehyde dehydrogenase) – have also been identified (Spector, 1998; Tsoy, Ravcheev, & Mushegian, 2009; Xu & Johnson, 1995; Walter, Ailion, & Roth, 1997; R. Khan & M. Spector, unpublished data). What is interesting is that these transport and/or utilization systems are being induced in the absence of exogenous (supplied) substrates. Thus, at least, some of these gene products likely play a role in C-starvation avoidance by scavenging for potential alternative carbon-energy sources should they become available (Spector, 1998; M. Antonio, S. Lucchini, R. Chaudhuri, A. Thompson, J. Hinton, M. Spector, & M. Pallen, unpublished data). Worthy of note is that the genes for propanediol utilization are also expressed under *in vivo*-mimicking conditions and are proposed to be involved in *S.* Typhimurium pathogenesis (Adkins et al., 2006; Heithoff et al., 1999; Sonck et al., 2009).

Additional genes shown to be CSI and involved in utilization of alternative C-sources are the *fad* genes. The *fad* gene products function in the degradation of fatty acids of a variety of chain lengths. The expression of the *fadA, fadB, fadD* and *fadI* genes were all increased in C-starved cells relative to non-starved cells according to microarray analysis (M. Antonio, S. Lucchini, R. Chaudhuri, A. Thompson, J. Hinton, M. Spector & M. Pallen, unpublished data). The *fadF* (or more accurately *fadE*; Campbell & Cronan, 2002) gene, encoding a fatty acyl-CoA dehydrogenase (ACDH) required for the degradation of a broad range of fatty acid chain length molecules, is induced within the first few hours C-starvation. The *fadE* gene is negatively-regulated by FadR in log-phase cells and positively-regulated by cAMP:CRP and ppGpp, but not σ^S, in C-starved cells. Results with *fadF(E)* null mutants indicate that early and continued fatty acid degradation is essential for long-term C-starvation survival (Spector, DiRusso, et al., 1999). The *fad* gene up-regulation correlates with a relative decrease in long-chain monosaturated fatty acids and reduction in cell size during starvation (El-Khani & Stretton, 1981; Huisman et al., 1996). This suggests that phospholipids from the cell's membranes are taken up and used as carbon-energy sources to mount and sustain the SSR. Interestingly, the

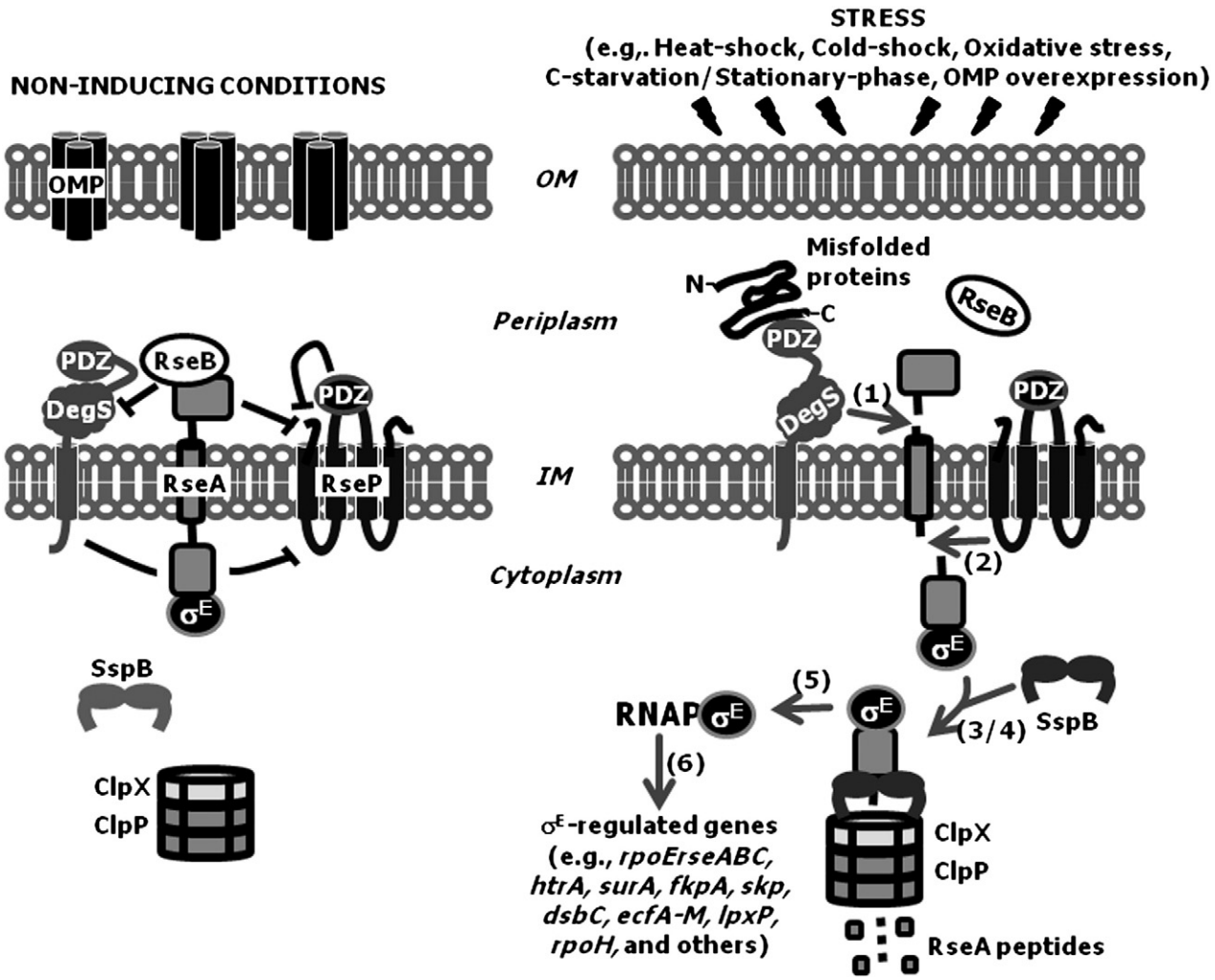

Fig. 2. Overview of σ^E activation/expression by regulated intramembrane proteolysis (RIP) and transcriptionl autoregulation in *Escherichia coli* and *Salmonella* (Ades, 2008; MacRitchie, Buelow, Price, & Raivio, 2008; Rowley et al., 2006). Under non-inducing conditions (left side) (*i.e.*, the absence of misfolded proteins) the PDZ domain of DegS and RseB inhibit DegS cleavage of RseA at its periplasmic cleavage site. When exposed to stresses that lead to the accumulation of misfolded proteins in the periplasm (right side), the DegS-PDZ domain binds to the exposed carboxy-terminal peptide (Y-x-F) of certain misfolded outer membrane proteins (OMPs; *e.g.*, OmpC). This binding plus the release of RseB from the periplasmic domain of RseA activates DegS protease activity leading to RseA cleavage at the periplasmic site (1). Relief of the DegS, RseA, and RseP-PDZ domain inhibition results in RseP protease activation, which cleaves RseA at the cytoplasmic cleavage site (2). This releases the σ^E-RseA inhibitory complex into the cytoplasm (3), which then binds to SspB, directing this complex to ClpXP for degradation (4). Proteolysis of the RseA fragment leads to σ^E release (5) allowing it to bind with core RNA polymerase (RNAP) and transcribe σ^E-dependent genes (6). The genes listed are examples of regulon members; see the corresponding text for additional genes and explanations. ⊢, indicates a negative effect on targeted step; ➔, indicates a positive effect on targeted step.

fadE gene is induced inside cultured MDCK epithelial cells but is not required for *S.* Typhimurium virulence in the mouse virulence model (Spector, DiRusso, et al., 1999).

The *dadAB* genes compose an operon and are needed for the utilization of L-/D-alanine and some other D-amino acids (*e.g.*, D-asparagine, D-phenylalanine, and D-methionine) as sole C-sources; *dadA* encodes a D-amino acid dehydrogenase and *dadB* encodes one (*i.e.*, catabolic) of two alanine racemases (McFall & Newman, 1996). The previously characterized (P- and) C-starvation inducible *stiB* locus (Seymour et al., 1996; Spector et al., 1988; Spector & Cubitt, 1992) turns out to be the *dadAB* operon (M. Pallen & M. Spector, unpublished data). A *dadAB* double mutant is defective in the utilization of L-alanine, D-alanine and D-alanyl-D-alanine as sole C-sources; however, a *dadA*$^+$*dadB* mutant is defective only in the utilization of L-alanine. Both *dadA-lac* and *dadB-lac* fusions where induced by L-alanine and C-starvation but not D-alanine or D-alanyl-D-alanine (N. Verneuil & M. Spector, unpublished data). A possible reason for the induction of *dadAB* by L-alanine and C-starvation is the utilization of L-alanine from protein degradation and D-alanine from peptidoglycan degradation, respectively, as C-sources. This is supported by the fact that the *dadAB* operon is required for long-term C-starvation survival. Surprisingly, the *dadAB* operon was also needed for adaptive H_2O_2-inducible H_2O_2 resistance (independent of OxyR) but not CSI cross-resistance to H_2O_2 (Seymour et al., 1996). The *dadAB* operon does not requires σ^S for its induction during C-starvation and is negatively regulated by cAMP receptor protein (CRP) but apparently independent

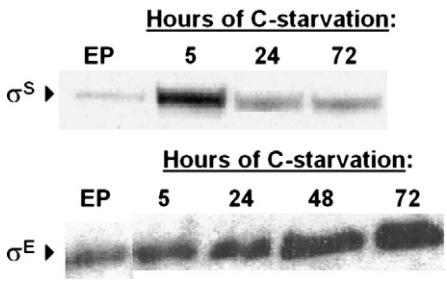

Fig. 3. Western blot hybridization showing accumulation of σ^S (top panel) and σ^E (bottom panel) in exponential-phase (EP) cells and cells glucose (C)-starved for up to 72 hours.
(W.J. Kenyon & M.P. Spector, unpublished data).

of cAMP during exponential-phase growth (O'Neal et al., 1994; Spector & Cubitt, 1992). These findings suggest that the ability to metabolize L-alanine/D-alanine (and/or possibly other D-amino acids) is an essential component of the SSR, especially with respect to long-term C-starvation survival. The sources of these substrates during C-starvation are likely either from the environment (possibly released from lysed/dead bacteria or other organisms) or the degradation of the bacteria's own peptidoglycan and/or proteins.

2.1.3. Expression of alternative (anaerobic) respiration systems

Nyström, Larsson, and Gustafsson (1996) proposed that C-starved cells growing aerobically exhibit physiologic characteristics of cells shifted to anaerobic conditions. Based upon the defective C-starvation survival phenotype of an *E. coli arcA* mutant, they proposed that C-starvation survival requires: (a) reduction in electron donor production, (b) diminished aerobic respiratory enzyme activity – thus, decreased reactive O_2 species generation limiting potential macromolecular damage – and (c) control of the rate of energy source utilization (Nyström et al., 1996). The proposed similarities between C-starved and anaerobically-growing cells including the documented reduction in aerobic respiratory enzymes in C-starved cells and expression of alternative respiratory enzymes that use alternative terminal electron acceptors (*e.g.*, fumarate, nitrate and nitrite) to produce utilizable energy during anaerobiosis, suggest that the utilization of alternative respiratory systems may be important in C-starved cells. However, the expression of alternative respiratory enzyme systems typically requires the particular electron acceptor to be present in the absence of oxygen (Gennis & Stewart, 1996). Nonetheless, the importance of alternative respiratory systems in (aerobically grown) C-starved cells is supported by the finding that previously described *stiA* mutations are in the *narZ* gene. The *narZ* gene is the second gene of the *narUZYWV* operon encoding a nitrite extruder/nitrate transporter protein (NarU) and a second, so-called cryptic, nitrate reductase NR-Z (Clegg, Jiam, & Cole, 2006; Spector, Garcia del Portillo, et al., 1999). NR-Z is nitrate-unresponsive and anaerobiosis-repressed. In addition to being C-starvation-inducible, the *narUZYWV* operon is P- and N-source starvation-inducible. Furthermore, *narUZYWV* expression is σ^S-dependent during starvation, cAMP-CRP repressed during exponential aerobic growth, FNR-repressed during anaerobic growth and partially repressed by reduced-form of OxyR ($OxyR_{red}$) during exponential aerobic growth (Gennis & Stewart, 1996; O'Neal et al., 1994; Seymour et al., 1996; Spector, 1990; Spector et al., 1988; Spector & Cubitt, 1992; Spector, Garcia del Portillo, et al., 1999). Thus, σ^S accumulation in C-starved cells overcomes the cAMP-CRP repression and aerobic growth relieves the FNR-repression allowing for increased expression of *narUZYWV*. Furthermore, when exponential cells growing aerobically are adapted with sub-inhibitory levels of H_2O_2, *narUZYWV* becomes partially derepressed as $OxyR_{red}$ becomes oxidized. In agreement with its complex regulation by known stress regulators, NR-Z is essential for long-term C-starvation survival, CSI cross-resistance to high temperature and acid pH as well as H_2O_2-inducible adaptive H_2O_2 resistance (Seymour et al., 1996; Spector & Cubitt, 1992; Spector, Garcia del Portillo, et al., 1999). Clegg et al. (2006) showed that in *E. coli* NarU accumulates in C-starved and chemostat-slow growing cells (with or without nitrate) and provides a selective advantage in the absence of the nitrate-inducible anaerobiosis-inducible NarK nitrate transporter to cells during slow-growth and starvation conditions. However, their studies were carried out in the presence of (albeit limiting) nitrate concentrations. The role of NR-Z in C-starved cells and H_2O_2-adapted cells is not clear. Based on the phenotypes associated with null mutations, it appears to involve more than its function as a nitrate reductase, since no exogenous nitrate is provided under the conditions of these studies (Seymour et al., 1996; Spector & Cubitt, 1992; Spector, Garcia del Portillo, et al., 1999). This raises the possibility that NR-Z may be important for the defense against "aging" as proposed by Nyström et al. (1996). Interestingly, *narZ* is induced approximately twenty-fold within MDCK cells and a *narZ* null mutant is attenuated about ten-fold in the BALB/c mouse model (Spector, Garcia del Portillo, et al., 1999).

According to microarray analyses, several other alternative respiration systems are up-regulated in aerobically grown C-starved cells (M. Antonio, S. Lucchini, R. Chaudhuri, A. Thompson, J. Hinton, M. Spector, & M. Pallen, unpublished data). Again, the increased expression of these systems occurs independently of their substrates/electron acceptors. These include: (a) a known (*dmsABC*) and three putative (STM1499-1496; STM2530-2528; STM4305-4307) anaerobic dimethyl sulfoxide (DMSO) reductases, (b) a tetrathionate reductase (*ttrAB; ttrSR* TCS), (c) a nitrate-inducible formate dehydrogenase (*fdnIHG*), (d) hydrogenase 1 complex/cytochrome oxidase (STM1786-1793), (e) hydrogenase 3 complex (*hydN hycABCDEFGHI*) — part of the formate-hydrogen lyase complex, (f) a periplasmic nitrate reductase (*napFDAGHBC*), (g) a nitrite reductase (*nirBD*), (h) a (formate-dependent) nitrite reductase (*nrfABCDG*), (i) an anaerobic sulfide reductase (*asrABC*), and (j) a trimethylamine *N*-oxide (TMAO) reductase (*torCAD*).

2.1.4. Modification of cell envelope structures

Another locus identified as a C-starvation-inducible SSR locus is *stiC* (O'Neal et al., 1994; Seymour et al., 1996; Spector, 1990; Spector et al., 1988; Spector & Cubitt, 1992). The *stiC* locus was found to encode the two gene operon *yohC pbpG* (Kenyon et al., 2007). The *yohC* gene encodes a 195 amino acid protein with an N-terminal cytoplasmic domain containing six histidine residues (multiple histidine residues is a conserved motif among the YohC orthologues in the γ-proteobacteria) and five predicted transmembrane domains supporting its proposed function as an inner membrane transport protein. An *E. coli yohC* mutant fails to reduce selenate to selenium but is able to reduce selenite to elemental selenium; thus, YohC was proposed to be involved in selenate transport (Bébien et al., 2002). However, *S.* Typhimurium *yohC* mutants are not defective in the reduction of either selenate or selenite to elemental selenium suggesting a divergent function for YohC or compensatory functions that compensate for the lack of YohC in selenate transport (Kenyon et al., 2007). The *pbpG* gene encodes the periplasmic DD-endopeptidase penicillin-binding protein (PBP) 7/8 (Henderson, Dombrosky, & Young, 1994; Henderson, Templin, & Young, 1995; Romeis & Höltje, 1994). The *yohC pbpG* operon is not only C-starvation-inducible but is P and N-starvation induced as well. Its expression is σ^S-dependent, positively regulated by ppGpp, and cAMP-CRP repressed during exponential-phase growth (O'Neal et al., 1994; Seymour et al., 1996; Spector & Cubitt, 1992). Kenyon et al. (2007) later showed that strains lacking *yohC* and unable to induce *pbpG* during the first five hours of C-starvation are defective in CSI cross-resistance to H_2O_2 and 55 °C. In comparison, strains lacking *yohC* but able to induce *pbpG* in five-hour C-starved cells exhibit wild type CSI cross-resistance to H_2O_2 and 55 °C. Interestingly, CSI levels of *pbpG* are not essential for resistance to these stresses in cells C-starved for twenty-four hours. Mutants unable to induce *pbpG* during the first few hours of C-starvation are also defective in C-starvation survival (Kenyon et al., 2007; Spector & Cubitt, 1992). This indicates that YohC is not required for the SSR but CSI levels of *pbpG* are required for the SSR in five hour, but not twenty-four hour, C-starved cells. The need for PBP 7 early during the SSR may reflect its proposed functions in peptidoglycan remodeling and/or changes in cell shape (Meberg, Paulson, Priyadarshini, & Young, 2004; Romeis & Höltje, 1994) since both these events occur early during C-starvation. Additionally, based upon the profile of β-lactam antimicrobics (those able to lyse non-growing cells) that bind to it, PBP 7 is proposed to have a role in inhibiting autolysis of non-growing *E. coli* cells by helping to produce an autolysis-resistant peptidoglycan (Tuomanen & Schwartz, 1987). A logical conclusion from this is that induced levels of PBP 7 are required to generate an appropriately structured peptidoglycan that affords the cell resistance (perhaps by inhibiting autolysis) to the effects of C-starvation and oxidizing agents or high temperature early during C-starvation. An interesting addition to this story is that the *yohC pbpG* operon is induced more than 12-fold within MDCK epithelial cells;

although, this does not correspond to an overt reduction in virulence in the BALB/c mouse model (Kenyon et al., 2007).

Another putative C-starvation-inducible gene whose protein product modifies structures in the cell envelope is the *lpxP* (*ddg*) gene. In *E. coli*, this gene encodes a cold-shock inducible palmitoleoyl transferase that adds a palmitoleoyl fatty acyl group to the lipid A of LPS. The addition of palmitoleoyl acyl groups to lipid A is thought to increase the fluidity of the outer membrane (Carty, Sreekumar, & Raetz, 1999). The *lpxP* gene is also regulated by σ^E in *E. coli* (Rezuchova, Miticka, Homerova, Roberts, & Kormanec, 2003). The *Salmonella lpxP* homolog exhibits significant induction in C-starved cells based upon microarray analyses (M. Antonio, S. Lucchini, R. Chaudhuri, A. Thompson, J. Hinton, M. Spector, & M. Pallen, unpublished data). However, preliminary results indicate that a *lpxP* null mutant was not significantly defective in the SSR compared to wild type cells (L. Pham and M.P. Spector, unpublished data).

2.2. Acid stress

Acid tolerance or resistance is a common and important environmental stress encountered by *Salmonella* serovars. The development of acid tolerance/resistance in *S. enterica* is very complex and highly influenced by stage of growth (*e.g.*, exponential-phase versus stationary-phase), growth temperature, the mode of environmental acidification (*e.g.*, the presence of organic or fatty acids versus inorganic acids) and/or the presence of certain amino acids (*e.g.*, arginine, lysine) (Álvarez-Ordóñez, Fernández, Bernardo & López, 2010a, 2010b; Audia, Webb, & Foster, 2001; Karatzas et al., 2008; Kieboom & Abee, 2006; Kwon & Ricke, 1998; Xu, Lee, & Ahn, 2008).

Acid resistance is especially important for foodborne pathogens that must survive the acidic pH of the stomach – which can drop to around pH 2 under fasting conditions – before entering and colonizing the small intestines or colon (Berk, de Jonge, Zwietering, Abee, & Kieboom, 2005; Lin, Lee, Frey, Slonczewski, & Foster, 1995). For facultative intracellular pathogens like *S.* Typhimurium, acidification of the phago-lysosome creates another obstacle to survival in the host (Oh et al., 1996; Rathman, Sjaastad, & Falkow, 1996). Resistance to stomach acidity significantly effects the infectious dose of pathogens in food, *e.g.*, the infectious doses of *Vibrio cholerae*, non-Typhi *Salmonella*, and *Shigella flexneri* are approximately 10^9, 10^5 and 10^2 bacteria, respectively. Not surprisingly, this correlates strongly to the relative acid sensitivities of these bacteria with *V. cholerae* being very acid sensitive, *S. flexneri* being strongly acid resistant and *Salmonella* falling somewhere in between (Audia et al., 2001; Lin et al., 1995).

Acid stress is also frequently encountered naturally in many foods, such as fruit juices; or, as a result of the use of weak organic acids or short-chain (volatile) fatty acids (SCFA; *e.g.*, acetic acid, citric acid, propionic acids) as food preservatives (Álvarez-Ordóñez et al., 2010a; Álvarez-Ordóñez, Fernández, Bernardo, & López, 2009; Baik, Bearson, Dunbar, & Foster, 1996; Cherrington, Hinton, Mead, & Chopra, 1991; Jay, Loessner, & Golden, 2005; Kwon & Ricke, 1998). Thus, the ability to sense, respond and adapt to an acidified environment is key to the epidemiology and virulence of foodborne pathogens such as *S. enterica*.

Salmonella serovars are neutralophilic bacteria that have evolved multiple tolerance or resistance mechanisms to promote their survival during exposures to the normally lethal pHs of 3.0 or 2.5; these are generally referred to as acid tolerance responses (ATRs) and acid resistance (AR) mechanisms, respectively. Which system(s) plays the dominant role(s) depends on: (a) the phase of growth of the cells when the ATR is elicited [log-phase (LP) ATR versus stationary-phase (SP) ATR]; and/or (b) whether certain amino acids are present during exposure to the acidic pH of 2.5 (arginine- or lysine-dependent AR systems); and/or (c) whether acidification of the environment results from inorganic or organic acids (Álvarez-Ordóñez et al., 2010a, 2010b; Audia et al., 2001; Park, Bearson, Bang, Bang, & Foster, 1996).

2.2.1. Log-phase acid tolerance responses (ATR)

The LP ATR is elicited by exposing exponential-phase cells to a moderate (non-lethal) external pH (pH_{ex}) of 4.5–5.8 for, at least, one hour. Adaptation at pH_{ex} 5.8 induces a pH homeostasis system, which functions to maintain an internal pH (pH_{in}) that allows for the maintenance of cell viability when the external pH falls to 3.0 (Foster & Hall, 1991). Lowering the adapting pH_{ex} to 4.5 induces the expression of some fifty acid shock proteins (ASPs) (Audia et al., 2001; Foster, 1991, 1993, 1999; Foster & Hall, 1990; Foster & Moreno, 1999). Both of these adaptation processes allow for the survival of cells when subsequently exposed to the lethal pH_{ex} of 3.0 compared to unadapted cells that go from pH 7.5–7.7 directly into pH 3.0 media. Induction of the LP ATR not only results in increased acid resistance but it also imparts cross-resistance to other stresses, *e.g.*, oxidative stress, DNA damage, shifts to high or cold temperature, or high osmolarity (Foster & Hall, 1990; Lee, Lin, Hall, Bearson, & Foster, 1995; Leyer & Johnson, 1993; Xu et al., 2008).

The LP ATR in *S.* Typhimurium exhibits regulation by several regulatory proteins including: σ^S, Fur, and PhoP (Audia et al., 2001; Bearson, Benjamin, Swords, & Foster, 1996; Bearson, Wilson, & Foster, 1998; Foster, 1999; Hall & Foster, 1996; Lee et al., 1995; Park et al., 1998). The rapid acidification of exponential-phase cultures significantly increases σ^S levels in the cell largely because of increased translation of *rpoS* mRNA (Audia & Foster, 2003; Fig. 1). Under these conditions, σ^S levels are also controlled by inhibiting its proteolysis by the ClpXP proteasome, which is regulated by an atypical response regulator MviA (*a.k.a.*, RssB or SprE; Fig. 1). Strains lacking MviA exhibit increased levels of σ^S in exponential-phase due to protein stabilization with concomitant increases in σ^S-dependent gene expression and acid resistance (Bearson et al., 1996). At least ten of the fifty ASPs induced during the LP ATR are σ^S-dependent including OsmY (a putative periplasmic protein of unknown function) and SodCII (a periplasmic Cu^{2+}/Zn^{2+}-superoxide dismutase) (Fang et al., 1999). Both OsmY and SodCII are also induced during the SSR; and, therefore, may play a role in acid pH resistance generated during the LP ATR and SSR in *S. enterica*.

The *fur* gene product is a negative regulator of genes functioning in the assimilation or uptake of exogenous iron. As a result, Fur controls the intracellular levels of iron and, therefore, its potential role in oxidative damage to cytoplasmic macromolecules, *e.g.*, DNA and proteins (Lee & Helmann, 2007; Touati et al., 2000; Zheng et al., 1999). Foster and Hall (Foster & Hall, 1992; Hall & Foster, 1996) reported that *fur* mutants are defective in generating a LP ATR and that Fur positively regulates, directly or indirectly, several ASPs in an iron-independent manner. These researchers (Hall & Foster, 1996) later demonstrated that Fur separately senses iron and H^+ ion (*i.e.*, acid pH) levels through histidine residues located in separate domains of the protein.

This same research group also demonstrated that PhoP, the response regulator component in a two-component regulatory system (TCS) with the sensor-kinase PhoQ, is an ASP. They further demonstrated that PhoPQ is yet another regulator in the LP ATR controlling the acidic expression of both PhoP and PhoQ and, at least, three other ASPs (Bearson et al., 1998). PhoQ is inactivated by extracellular Mg^{2+} ions and, thus, can be activated under low Mg^{2+} ion concentrations, autophosphorylating itself and transphosphorylating the PhoP response regulator, which in turn directly or indirectly regulates multiple sets of genes involved in Mg^{2+} homeostasis and virulence (Lucas & Lee, 2000; Prost & Miller, 2008; Soncini et al., 1996). The *phoPQ* operon is also induced by moderate acid pH even under high Mg^{2+} ion concentrations (Bearson et al., 1998; Fig. 4). This suggests that PhoQ might sense acidic pH via the effect of H^+ ions on the conformation of the Mg^{2+} ion-binding site, which in turn activates its histidine kinase activity and the ultimate phosphorylation of PhoP. The importance of PhoP-regulated gene expression in macrophage phago-lysosome survival and *Salmonella* virulence correlates well with its role as a TCS that senses and responds to H^+ and Mg^{2+} ions since these are probable *in vivo* relevant cues. *In vivo* relevance is supported by the finding that phago-lysosome acidification – and, thus, PhoQ/P activation – is essential

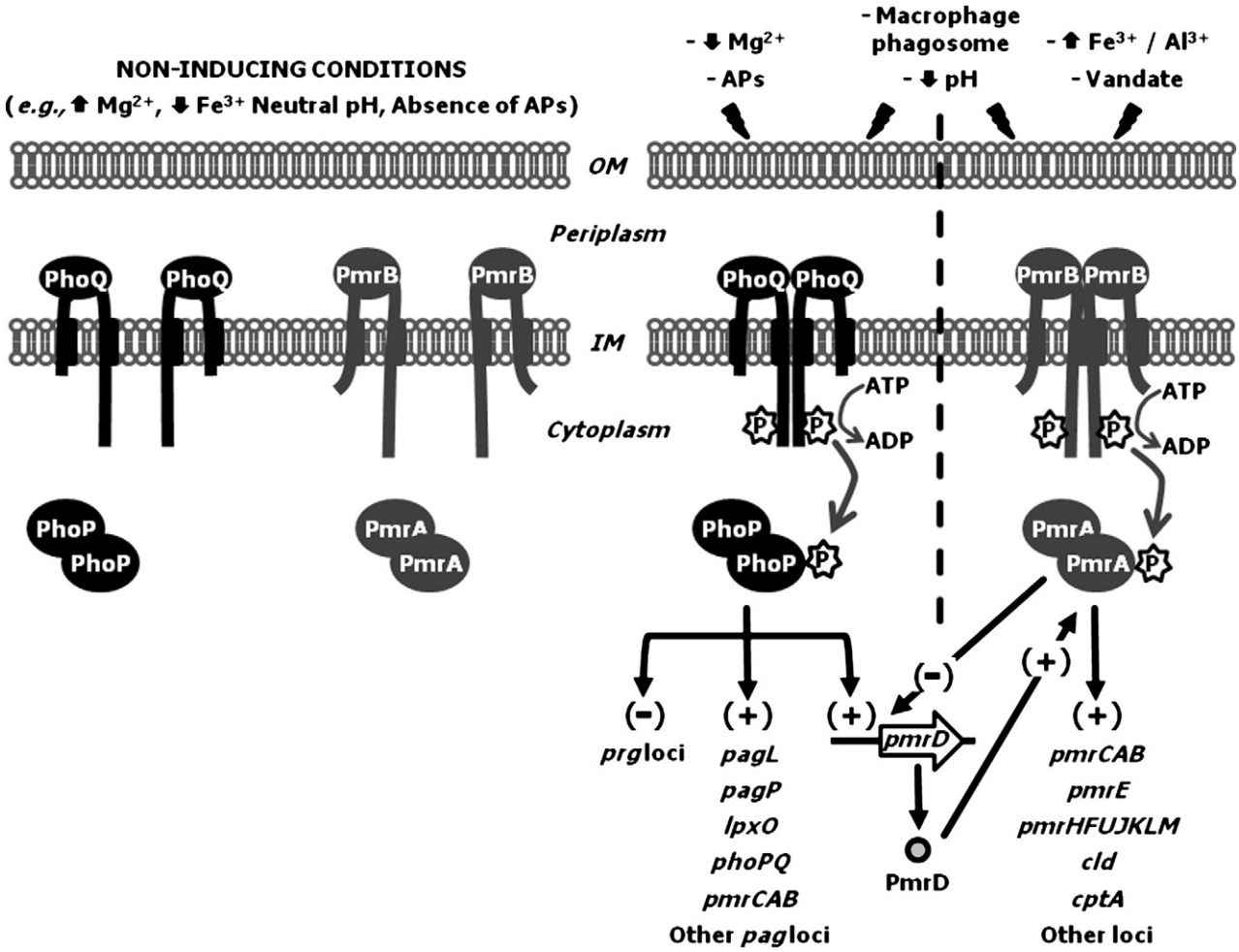

Fig. 4. Overview of the regulation of the PhoPQ and PmrAB systems in *Salmonella* (Ernst et al., 2001; Gunn, 2008). The PhoQ and PmrB sensor (histidine) kinases sense environmental cues such as Mg^{2+} ion concentration (PhoQ), Fe^{3+} ion concentrations (PmrB), acid pH or presence of antimicrobial peptides (APs). Under low Mg^{2+} levels, acid pH or the presence of APs, the PhoQ's his kinase is activated resulting in its autophosphorylation with subsequent transfer of the phosphoryl group to a conserved aspartate residue in PhoP forming PhoP-P. Similarly, under high Fe^{3+} or Al^{3+} concentrations, acid pH or presence of vandate, the PmrB his kinase is activated ultimately resulting in the phosphorylation of a conserved aspartate residue in PmrA resulting in PmrA-P. PhoP-P and PmrA-P both go on to regulate the transcription of genes required for AP resistance, acid resistance and survival within macrophages along with other functions. The PhoP-activated *pmrD* gene product PmrD connects PhoPQ activation to up-regulation of the PmrA-P regulon by binding to PmrA-P, thus stabilizing it and promoting PmrA-P-regulated transcription. The genes listed are examples of regulon members; see the corresponding text for additional genes and explanations. (+) indicates a positive effect (*e.g.*, up-regulation), while (−) indicates a negative effect (*e.g.*, down-regulation).

for *S.* Typhimurium survival within macrophages (Alpuche-Aranda et al., 1992; Rathman et al., 1996).

2.2.2. Stationary-phase ATR

Although starved or stationary-phase cells exhibit resistance to a challenge of pH_{ex} 3.0, this resistance declines as the time of exposure increases. However, if stationary-phase cells are first adapted to pH_{ex} 4.5 then they survive for much longer periods at pH_{ex} 3.0; this is called the stationary-phase (SP) ATR. Interestingly, acid pH-inducible SP ATR is σ^S-, Fur- and PhoP-independent indicating that it is distinct from the LP ATR (Lee et al., 1994). Bang et al. (2000) later showed that null mutations in the *ompR* gene made stationary-phase cells acid sensitive and unable to elicit a SP ATR. The *ompR* gene forms an operon with *envZ* in *S.* Typhimurium and other enterobacteria. The OmpR protein is the response regulator in a TCS with the EnvZ sensor-kinase known to sense and respond to changes in osmolarity (Mizuno & Mizushima, 1990; Pratt & Silhavy, 1996; Poolman et al., 2002; Wood, 2006, 2007). Thus, in addition to its role in osmoregulation, phosphorylated OmpR (OmpR-P) is a key regulator of the SP ATR (Fig. 5).

OmpR/EnvZ, like PhoPQ, is also needed for the expression of *Salmonella* pathogenicity island (SPI)-2 genes (Fass & Groisman, 2009; Lucas & Lee, 2000). Foster and colleagues (Bang et al., 2000; Bang et al., 2002) discovered that OmpR is an ASP and autoregulates the *ompR envZ* operon in response to acid pH. The acid pH induction of *ompR envZ* transcription requires EnvZ; but, the alternative phosphodonor molecule acetyl-phosphate (Ac-P) is needed for the induction of essential OmpR-dependent ASP gene expression, since *ack pta* double mutants (unable to make Ac-P) induce *ompR* expression but fail to generate a SP ATR (Audia et al., 2001; Fig. 5). The signal that EnvZ senses during acid shock conditions is not clear. However, it is proposed that Ac-P functions to phosphorylate OmpR as its levels increase in response to pH-mediated changes in DNA supercoiling around the *ompR* promoter region allowing OmpR-P to displace H-NS (a nucleoid binding protein) relieving its repression of *ompR* transcription (Audia et al., 2001). It is interesting to note that the RNAP-associated protein SspA plays a role in stationary-phase acid resistance through downregulation of *hns* expression in *E. coli* (Hansen et al., 2005); although, it is not clear if a similar scenario occurs in *Salmonella*.

2.2.3. Amino acid-dependent acid resistance (AR) mechanisms

Stationary-phase *S.* Typhimurium also expresses AR mechanisms that require the presence of either arginine or lysine for survival at pH_{ex}

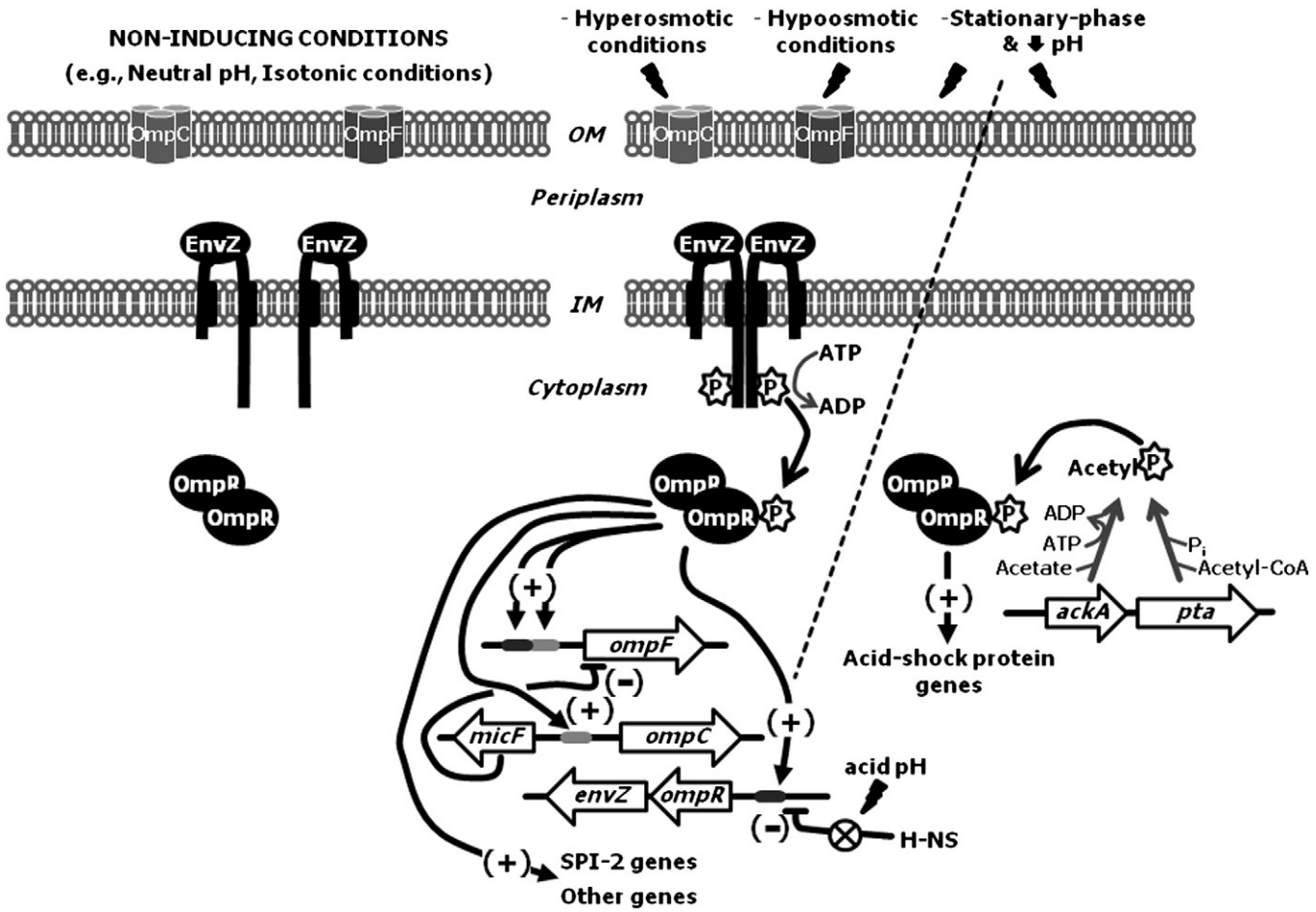

Fig. 5. Overview of the regulation of the EnvZ-OmpR system in *Salmonella* (Wood, 2006). The EnvZ sensor (his) kinase senses extremes in osmolarity and possibly acid pH. Under hyperosmotic stress, EnvZ his kinase is activated resulting in its autophosphorylation with subsequent transfer of the phosphoryl group to a conserved aspartate residue in OmpR forming OmpR-P. High levels of OmpR-P can bind to low affinity binding sites (*light gray bars*) upstream of the *ompC*, *micF* and *ompF* genes; however, *micF* RNA can bind to *ompF* mRNA inhibiting its translation. As a result, OmpC expression is promoted under hyperosmotic conditions relative to OmpF expression. In contrast, low osmolarity reduces the amount of OmpR-P so that OmpR-P primarily binds to a high affinity binding site (*dark gray bar*) upstream of *ompF*; thus, OmpF is preferentially expressed under hypoosmotic stress relative to OmpC. In stationary-phase pH-adapted cells, OmpR can be phosphorylated by either EnvZ or acetyl phosphate (produced by the products of the *ackA* or *pta* genes). However, acetyl phosphate plays a significant role in OmpR phosphorylation only when OmpR levels increase to high levels due to the effect of low pH in relieving the H-NS repression of the *ompR-envZ* promoter region. OmpR-P can then go on to induce a subset of acid-shock genes/proteins, which includes the *ompR-envZ* operon. The genes listed are examples of regulon members; see the corresponding text for additional genes and explanation. (+) indicates a positive effect (*e.g.*, up-regulation), while (−) indicates a negative effect (*e.g.*, down-regulation).

2.5. Typically, the SP ATR does not provide significant resistance to pH_{ex} 2.5. However, the *adiA*, *cadA* and *cadB* genes – encoding arginine decarboxylase, lysine decarboxylase and the lysine-cadeverine antiporter, respectively – all increase in cells adapted at pH_{ex} 4.5 in rich (Brain–Heart Infusion; BHI) medium. This correlates to an increase in acid resistance in pH_{ex} 4.5-adapted stationary-phase cells when challenged at pH_{ex} 2.5 in the presence of either arginine or lysine (Álvarez-Ordóñez et al., 2010b; Park et al., 1996). Kieboom and Abee (2006) had previously shown that the addition of arginine increased survival at pH_{ex} 2.5 only in stationary-phase cells grown under anoxic conditions as compared to microaerobic or aerobic growth conditions. These researchers also found that transcription of *adiA* and *adiC* (encodes the arginine-agmatine antiporter) is dependent on AdiY, acid pH and anaerobiosis (Kieboom & Abee, 2006). It is not clear whether these findings are in complete agreement with those of Álvarez-Ordóñez et al. (2010b) since it is unclear whether the cultures in the later study were grown under similar conditions. In any case, it is clear that the presence of arginine or lysine during extreme acid stress conditions (*e.g.*, in the stomach) may enhance the likelihood of survival of *S. enterica* serovars allowing them to enter and colonize the intestines. A similar case can be made for survival of *Salmonella* in very acidic foods.

2.2.4. Weak organic acid ATR

The manner in which the external milieu is acidified significantly influences the relative roles of acid tolerance mechanisms. Weak organic acids or short-chain fatty acids (SCFAs) can, in addition to acidifying the cytoplasm (*i.e.*, lowering the pH_{in}), also accumulate as an intracellular anion (Baik et al., 1996; Cherrington et al., 1991); while inorganic acid (*i.e.*, HCl) generally only acidifies the pH_{in} (Bearson et al., 1998; Park et al., 1996). Mechanisms of acid tolerance to organic and inorganic acids appear to be different; however, adaptation with inorganic acids provides resistance to acid stress mediated by organic acids or SCFAs, and vice versa (Baik et al., 1996; Bearson et al., 1998; Kwon & Ricke, 1998). Adaptation with SCFAs, in contrast to HCl, of exponential-phase cells is σ^S- and PhoP-independent; but, is Fur-dependent (Audia et al., 2001; Bearson et al., 1998; Foster, 1999). This is interesting since adaptation using either inorganic or organic acids leads to increased levels of σ^S (Audia & Foster, 2003; Baik et al., 1996; Bearson et al., 1996).

In a recent report, Álvarez-Ordóñez et al. (2010a) showed that growth temperature has a significant effect on the magnitude of the ATR in response to organic acids. Acid resistance was lowest in cells grown at 10 °C and increased with rising growth temperatures peaking at 37 °C

before falling off as the growth temperature increased to 45 °C (acid resistance was about the same in cells grown at either 25 °C or 45 °C). These researchers also demonstrated that adaptation with citric acid (as opposed to lactic acid or acetic acid) generated the strongest ATR in cells grown at 25 °C or 37 °C (Álvarez-Ordóñez et al., 2010a). Thus, growth temperature is an important parameter in generating acid resistance in *S.* Typhimurium strains influencing survival of *Salmonella* serovars in foodstuffs.

2.3. Oxidative stress

Salmonella serovars are exposed to a multitude of oxidizing agents as they pass through both host and non-host environments. In the presence of oxygen, reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) originate either from endogenous aerobic metabolism or as part of an assault from (phagocytic) cells of the immune system (Farr & Kogoma, 1991; Foster & Spector, 1995; Hassett & Cohen, 1989; Imlay, 2008; Janssen et al., 2003). These compounds react strongly with organic molecules causing damage to nucleic acids, proteins, lipids, and carbohydrates (Cabiscol et al., 2000; Farr & Kogoma, 1991; Imlay, 2008), and without protective mechanisms, bacterial cells would quickly succumb to an accumulation of oxidative damage. Consequently, *Salmonella* serovars have evolved several sophisticated molecular strategies for combating oxidative stress arising from a variety of sources.

In *Salmonella* (and *E. coli*), there are two major stress response pathways responsible for dealing with oxidative stress. The OxyR regulon responds specifically to the presence of H_2O_2, whereas the SoxRS regulon senses changes in the cellular redox state caused by redox-cycling agents (Christman et al., 1985; Morgan et al., 1986; Pomposiello & Demple, 2000; Pomposiello & Demple, 2001; Storz & Imlay, 1999; Storz, Tartaglia & Ames, 1990a; Tsaneva & Weiss, 1990). Although a few genes are under the control of both regulons, there is surprisingly limited overlap between the OxyR and SoxRS pathways, perhaps reflecting different types of oxidative damage caused by H_2O_2 and redox-cycling agents.

2.3.1. OxyR-regulated oxidative stress responses

Exposure to H_2O_2 results in an increased synthesis of over forty proteins (Christman et al., 1985; Demple & Halbrook, 1983; Morgan et al., 1986; VanBogelen et al., 1987). The expression of, at least, ten of these proteins is controlled by the H_2O_2-responsive transcriptional regulator OxyR (Pomposiello & Demple, 2001; Storz & Imlay, 1999; Storz et al., 1990a; Storz, Tartaglia & Ames, 1990b). OxyR protein forms a homotetramer, with each OxyR subunit containing a pair of cysteine residues that function in sensing oxidative stress (Storz & Zheng, 2000; Storz et al., 1990a; Storz et al., 1990b; Zheng et al., 1998). Levels of H_2O_2 or redox state of the cell appear to act as a molecular switch that causes conformational change(s) in OxyR promoting its role as a transcriptional activator (Helmann, 2002; Kim et al., 2002; Storz & Zheng, 2000). The oxidized form of OxyR ($OxyR_{ox}$) induces the transcription of several genes which function in the breakdown of H_2O_2 (*katG*), protection of DNA (*dps*), reduction of oxidized lipids (*ahpCF*), and formation of disulfide bonds (*gorA*, *grxA*, and *trxC*) (Storz & Zheng, 2000). In addition, $OxyR_{ox}$ activates *fur* gene expression and the expression of a small regulatory RNA (sRNA) OxyS (Altuvia et al., 1997; Zheng et al., 1999). Increased Fur levels are proposed to prevent the formation of hydroxyl radicals via the Fenton reaction by reducing iron uptake. The OxyS sRNA functions in preventing mutagenesis under conditions of oxidative stress and may be a regulatory link between the OxyR regulon and other stress response networks, *e.g.*, the σ^S regulon (Cadenas, 1989; Storz & Zheng, 2000; Zheng et al., 1999). The reduction of OxyR ($OxyR_{red}$) is catalyzed by the OxyR-regulated GrxA (glutaredoxin 1) using glutathione as the reductant (Zheng et al., 1998). $OxyR_{red}$ autorepresses *oxyR* gene transcription (Storz & Zheng, 2000; Zheng et al., 1998). Another locus repressed by the $OxyR_{red}$ is the *narUZYWV* operon of *S.* Typhimurium (Seymour et al., 1996; Spector, Garcia del Portillo, et al., 1999).

2.3.2. SoxRS-regulated oxidative stress responses

In *S.* Typhimurium and *E. coli*, exposure to redox-cycling compounds up-regulates the expression of greater than 100 proteins many of which are regulated by the SoxRS system (Greenberg & Demple, 1989; Greenberg et al., 1990; Pomposiello & Demple, 2000; Pompsiello et al., 2001; Tsaneva & Weiss, 1990; Walkup & Kogoma, 1989). It was initially believed that the SoxRS system functions in superoxide resistance since redox-cycling agents (*e.g.*, paraquat, phenazines, plumbagin, menadione, *etc.*) generate superoxide and some SoxRS regulon gene products function to detoxifiy (*e.g.*, *sodA*) or prevent production of superoxide (*e.g.*, *nfsA*). However, this does not appear to be the case, at least for *E. coli*, since the SoxRS system is not efficiently activated by even high levels of superoxide and overproduced superoxide dismutase (SOD) does not prevent activation by redox-cycling agents (Gu & Imlay, 2011). The SoxR protein forms a homodimer with each monomer containing an iron-sulfur center [2Fe–2S], which undergo oxidation in the presence of redox-cycling agents (Demple et al., 1999; Touati, 2000b). Gu and Imlay (2011) demonstrated that the 2Fe–2S clusters of SoxR are directly oxidized by redox-cycling agents and not by superoxide. The oxidation of SoxR results in a conformational change producing oxidized SoxR ($SoxR_{ox}$), which transcriptionally activates the *soxS* gene (Nunoshiba et al., 1992; Wu & Weiss, 1991). The transcriptional activator SoxS in turn induces the expression of over a dozen genes. In addition to *sodA* and *nfsA*, members of the SoxS regulon include: (a) *fldA*, *fldB* and *fpr*, maintenance of the reduced state of iron-sulfur centers, (b) *nfo*, repair of DNA damage, (c) *zwf*, increase of reducing power within the cell, (d) *fumC* and *acnA*, oxidation-resistant isoenzymes of fumarase and aconitase, and (e) *tolC*, *micF* and *acrAB*, exclusion or efflux of redox-cycling agents from the cell (Storz & Zheng, 2000). Like OxyR, the SoxRS system also induces expression of *fur*, preventing formation of hydroxyl radicals through the Fenton reaction (Zheng et al., 1999). In *E. coli* and presumably *Salmonella*, once the oxidative stress is alleviated, $SoxR_{ox}$ is reduced through the action of reducing systems encoded by *rseC* (the last gene of the *rpoE-rseABC* operon) and *rsxABCDGE* (Koo et al., 2003) and SoxS is proteolytically degraded stopping the response (Griffith et al., 2004). Although, defense against superoxide stress is part of the function of the SoxRS regulon it apparently is not the most important because redox-cycling agents are toxic even under anaerobic conditions. The defense against redox-cycling compounds – which are produced by a number of bacteria and plants to reduce competition (Inbaraj & Chignell, 2004; Turner & Messenger, 1986) – seems to be the primary function of the SoxRS regulon.

2.3.3. Roles of other regulators in oxidative stress responses

OxyR and SoxRS do not solely control all of the proteins that function in resistance to oxidative stresses. Other regulatory factors also control the expression of oxidative stress resistance genes, either alone or in addition to OxyR or SoxRS. For example, the genes *katE*, *xthA* and *sodC*, encoding hydroperoxidase II, exonuclease III and periplasmic superoxide dismutase, respectively, are regulated by the alternative sigma factor σ^S (Storz & Zheng, 2000). The genes *katG* (hydroperoxidase I), *gorA* (glutathione reductase), *dps* (DNA protection), *narZYWV* (nitrate reductase-Z) and *oxyR*, itself, are regulated by both σ^S and OxyR (Michán et al., 1999; Seymour et al., 1996; Spector, DiRusso, et al., 1999; Spector, Garcia del Portillo, et al., 1999; Storz & Zheng, 2000). Futhermore, the $OxyR_{ox}$-regulated small RNA OxyS represses *rpoS* translation under certain conditions (Altuvia et al., 1997; Zhang et al., 1998). The SoxRS-regulated *fumC* and *acnA* genes are also under σ^S control (Storz & Zheng, 2000). The oxygen-responsive regulators FNR and ArcAB also influence the expression of genes in the SoxRS regulon and a few genes not regulated by either SoxRS or OxyR (Fink et al., 2007; Lu et al., 2002; Storz & Zheng, 2000). In addition to σ^S-regulated responses, networks responsive to thermal stress, envelope stress and

starvation-stress, which are regulated by σ^H and σ^E (Bang et al., 2005; Kenyon et al., 2002), and the SOS response to extreme DNA damage, regulated by LexA and RecA (Buchmeier et al., 1993; Farr & Kogoma, 1991), include proteins functioning in resistance to oxidative stress. In fact, DNA repair mechanisms may be more important than catalase activity for *Salmonella* survival within the phagolysosome of macrophages (Buchmeier et al., 1995).

Henard et al. (2010) reported that the stringent response regulator DksA plays a role in antioxidant responses in *Salmonella*. In this study, the researchers demonstrate that DksA "boosts" antioxidant defenses in nutritionally stressed stationary phase cells. They propose that DksA does this through its control of redox balance. DksA can control redox balance through its affect on NAD(P)H/NAD(P)$^+$ levels via its regulation of steps within the pentose phosphate pathway and tricarboxylic acid cycle. These researchers also found that DksA promotes resistance to H_2O_2 and ROS generated by NADPH phagocyte oxidase (phox). They present the hypothesis that the control of central metabolic pathways (Spector, 2010) by DksA helps maintain redox homeostasis and that this is essential for antioxidant defenses in *Salmonella* and other bacteria.

Neither OxyR nor SoxRS are necessary for *S.* Typhimurium survival within murine macrophages or for full virulence in mice – unlike for *E. coli* – suggesting that there are compensatory mechanisms produced by *S.* Typhimurium involved in combating oxidative stress *in vivo* (Fang et al., 1997; Nunoshiba et al., 1993; Nunoshiba et al., 1995; Papp-Szabò et al., 1994; Taylor et al., 1998). In support of this idea, *S.* Typhimurium produces a number of H_2O_2 scavengers and periplasmic superoxide dismutase activities (SodCI and SodCII) that play a role in virulence (Battistoni, 2003; Craig & Slauch, 2009; Hébrard et al., 2009; Uzzau et al., 2002). A possible explanation for this is that salmonellae must deal with ROS (as well as RNS) generated both endogenously and externally during the respiratory burst of phagocytes. Interestingly, only SodCI is needed for virulence in mice presumably by protecting against extracytoplasmic damage caused by phagocyte-generated superoxide. This suggests that SodCII helps contend with oxidative stress in other environments (Craig & Slauch, 2009; Uzzau et al., 2002). Several transcriptional regulators in *S.* Typhimurium (*slyA*, *rpoS*, *rpoE* and *recA*) also control aspects of oxidative-stress resistance and virulence in mice (Buchmeier et al., 1993; Buchmeier et al., 1997; Fang et al., 1992; Testerman et al., 2002). Some of the individual genes controlled by these regulatory proteins could encode undiscovered *Salmonella*-specific antioxidant defenses involved in pathogenesis.

2.4. Thermal stress and envelope (extracytoplasmic) stress

High temperature stress has differing effects on the cytoplasm and cell envelope of Gram-negative bacteria. In *Salmonella* and *E. coli*, the response to thermal stress is accordingly subdivided into a cytoplasmic heat-shock response controlled by the classic heat-shock sigma factor σ^H and an extracytoplasmic response regulated by the extracytoplasmic function (ECF) sigma factor σ^E (Ades, 2008; Alba & Gross, 2004; Guisbert et al., 2008; Nonaka et al., 2006). Interestingly, several σ^H- and σ^E-regulated genes encoding cytoplasmic and periplasmic chaperones and proteases appear to contribute to *Salmonella* virulence (Conlin & Miller, 2000; Foster & Spector, 1995; Humphreys et al., 2003; Lewis et al., 2009; Rowley et al., 2010; Sydenham et al., 2000; Takaya et al., 2003; Takaya et al., 2004; Thomsen et al., 2002). The molecular mechanisms of σ^H and σ^E activation differ, but both mechanisms allow for a rapid response to thermal stress. Although, originally described for its role in bacterial responses to higher temperature heat-shock, σ^E was later shown to function in a number of responses to stresses, which result in the accumulation of misfolded envelope proteins, primarily proteins destined for the outer membrane (*i.e.*, envelope stress).

Over recent years, at least five systems have been found to play a role in bacterial envelope stress responses (ESRs) the σ^E, Cpx (*c*onjugative *p*ilus e*x*pression), phage-shock protein (Psp), Bae (*b*acterial *a*daptive *r*esponse) and Rcs (*r*egulator of *c*apsular *s*ynthesis) responses (Alba & Gross, 2004; Rowley et al., 2006; Ades, 2008; MacRitchie et al., 2008). The σ^E, Cpx and Psp ESRs are discussed below, while the Bae and Rcs ESRs are discussed under bile and AP resistance mechanisms, respectively, based on known functions in *Salmonella*.

2.4.1. σ^H-Regulated thermal stress responses

Transcription of the *rpoH* gene, encoding σ^H, depends very little on changes in temperature (Yura & Nakahigashi, 1999). Instead, the intracellular concentration of σ^H is regulated at the translational level. At lower temperatures (30 °C), intramolecular hydrogen bonding prevents translation of *rpoH* mRNA, while at elevated temperatures (42 °C), the secondary structure of *rpoH* mRNA opens allowing ribosomal binding and translation (Morita, Kanemori, et al., 1999; Morita, Tanaka, et al., 1999). This molecular switch acts as a built-in molecular thermometer for monitoring thermal stress. As part of the RNA polymerase holoenzyme complex (RNAPσ^H), σ^H directs the transcription of more than 30 heat-shock proteins (HSPs) which function as molecular chaperones and proteases (Foster & Spector, 1995; Guisbert et al., 2008; Lund, 2001; Nonaka et al., 2006). The cytoplasmic heat-shock response is down-regulated by σ^H-regulated proteases, *e.g.*, the membrane-bound ATP-protease FtsH (Herman et al., 1995; Kanemori et al., 1999; Morita et al., 2000; Straus et al., 1987; Tomoyasu et al., 1995). This negative feedback loop helps to control overexpression of HSPs. Formation of RNAPσ^H complex is also controlled by the competition between RNA polymerase and the σ^H-regulated DnaK-DnaJ chaperone complex for σ^H binding (Guisbert et al., 2004; Morita et al., 2000). The DnaK-DnaJ chaperone complex also binds to misfolded proteins, which would increase at higher temperatures allowing more RNAPσ^H binding and increased HSP expression. Therefore, σ^H is under control by the HSPs present in the cytoplasm and by the direct effect of temperature on *rpoH* translation and protein misfolding (Guisbert et al., 2004; Tomoyasu et al., 1998; Yura et al., 2000).

2.4.2. σ^E-Regulated thermal and envelope stress responses

While the σ^H-regulon largely deals with the accumulation of misfolded proteins in the cytoplasmic compartment, the alternative sigma factor σ^E, (encoded by *rpoE*), controls multiple cell envelope, or extracytoplasmic, functions that mitigate the effects (*e.g.*, accumulation of misfolded proteins) of high temperatures on the extracytoplasmic compartment as well as other factors causing envelope stress (Alba & Gross, 2004; Rowley et al., 2006; Ades, 2008; MacRitchie et al., 2008; Fig. 2). However, the fact that one of the promoters controlling expression of the *rpoH* gene are under σ^E control (Erickson & Gross, 1989; Wang & Kaguni, 1989) allows for a coordinated response to thermal, and possibly general envelope, stress that involves both cellular compartments.

At lower temperatures, σ^E is sequestered by the membrane-bound anti-sigma factor RseA (De Las Peñas et al., 1997b; Missiakas et al., 1997). At temperatures above 45 °C, misfolded proteins, destined for the outer membrane and/or periplasm, accumulate in the periplasm triggering the sequential proteolytic cleavage of RseA by the inner membrane proteases DegS and RseP (YaeL). This regulated intramembrane proteolysis (RIP) of RseA results in the release of an σ^E-RseA inhibitory complex into the cytoplasm. Interaction with SspB directs this complex to the ClpXP proteasome; resulting in the degradation of the RseA fragment and release of σ^E into the cytoplasm (Alba & Gross, 2004; Rowley et al., 2006; Ades, 2008; MacRitchie et al., 2008; Fig. 2). Once released σ^E forms a complex with RNA polymerase (RNAPσ^E) and directs the transcription of specific sets of genes whose protein products play a role in combating extracytoplasmic stress. Members of the σ^E regulon include: periplasmic chaperones (*e.g.*, SkpA, SurA, DegP), peptidyl-prolyl isomerases (*e.g.*, SurA, FkpA), (serine) proteases (*e.g.*, DegP) and other factors involved in outer membrane biosynthesis and function (Dartigalongue et al., 2001; MacRitchie et al., 2008; Rhodius et al., 2006; Rowleyet al., 2006; Skovierova et al., 2006). Because the *rseA* gene is part of the *rpoE* operon (*rpoE rseABC*), which is itself σ^E-

regulated, the extracytoplasmic response to thermal stress is feedback controlled. As intracellular σ^E levels rise in response to extracytoplasmic stress, so does the concentration of the anti-sigma factor RseA in the inner membrane replenishing this key "stop" signal for the response (Dartigalongue et al., 2001; Skovierova et al., 2006).

Several facts suggest that the σ^E regulons of *Salmonella* and *E. coli* have diverged over time. For example, *rpoE* null mutants cannot be isolated in *E. coli* without suppressor mutations (Button, Silhavy, & Ruiz, 2006; De Las Peñas, Connolly, & Gross, 1997a). However, *rpoE* null mutants of serovar Typhimurium are viable although they can exhibit some growth defects (Humphreys et al., 1999). Furthermore, *Salmonella rpoE* mutants show impaired thermotolerance as well as decreased survival in macrophages and attenuated virulence in mice (Humphreys et al., 1999; Testerman et al., 2002). In addition, there are some differences in the regulation of certain genes involved in thermotolerance, such as *degP* (*htrA*), *fkpA* and *surA*, and in the consensus promoter sequences recognized by σ^E in *Salmonella* and *E. coli* (Dartigalongue et al., 2001; Kenyon et al., 2010; Lewis et al., 2009; Rhodius et al., 2006; Skovierova et al., 2006). These variations may be related to particular environmental adaptations these bacteria developed as they evolved (Winfield & Groisman, 2003).

A number of σ^E- and σ^H-regulated genes are involved in *Salmonella* virulence. Null mutations in the σ^E-regulated *degP (htrA)* gene (encodes a periplasmic serine protease) reduce survival of *S.* Typhimurium inside murine macrophages and attenuate virulence in mice (Sydenham et al., 2000). Inactivation of the σ^E-regulated *fkpA* gene (encodes a periplasmic, peptidyl-prolyl isomerase) alone has very little effect on the survival of *S.* Typhimurium inside macrophages or on virulence in mice, but has a significant effect when combined with a *surA* or *degP* null mutation (Humphreys et al., 2003). Both *degP* and *surA* null mutant strains exhibit potential as live oral vaccines against salmonellosis. The σ^H-regulated genes *dnaK*, *dnaJ*, *groE*, *clpP*, *lon*, and *opdA* also appear to be involved in *Salmonella* virulence (Conlin & Miller, 2000; Foster & Spector, 1995; Takaya et al., 2003, 2004; Thomsen et al., 2002). In addition, derepression of a number of *Salmonella* genes during a temperature upshift from 25–37 °C is dependent on the histone-like DNA-binding protein H-NS (Fang & Rimsky, 2008; Ono et al., 2005). Together, this suggests that these cytoplasmic and periplasmic chaperones and proteases play a key role in combating protein-misfolding inducing stresses encountered within host environments.

2.4.3. CpxRA-regulated envelope stress response systems

The σ^E regulon works in coordination with the two-component CpxRA system in controlling the expression cell envelope factors needed to prevent or repair damage caused by high temperature or other factors causing envelope stress (Becker et al., 2005; Darwin, 2005; MacRitchie et al., 2008; Rowley et al., 2006; Ruiz & Silhavy, 2005). The Cpx system is best described in *E. coli* where is it shown to control the expression of 100–200 proteins (many of which overlap with the σ^E-regulon) in response to various conditions believed to result in envelope or extracytoplasmic stress (De Wulf et al., 2002; MacRitchie et al., 2008; Price & Raivio, 2009; Raivio & Silhavy, 1999; Rowley et al., 2006; Ruiz & Silhavy, 2005). The Cpx system down-regulates both *rpoH* and *rpoE(rseABC)* genes and up-regulates it own expression (*cpxRA* operon) and *cpxP* (encodes a periplasmic negative regulator of CpxA sensor-kinase activity). Among the genes/operons up-regulated by CpxR-P are those encoding periplasmic proteins possessing protease (*e.g.*, *htrA* or *degP*), chaperone (*e.g.*, *skp*, *htrA)*, protein disulfide isomerase (*e.g.*, *dsbA*) and peptidyl-prolyl isomerase (PPIase; *e.g.*, *ppiA*, *ppiD*) activities. Cpx-mediated regulation also seems to overlap with the EnvZ-OmpR and BaeSR (discussed later) regulons. For instance, CpxR-P up-regulates *ompC* and down-regulates *ompF* expression and appears to increase BaeR up-regualtion of the *acrD* and *mdtABCD* genes encoding multidrug efflux/transporter systems.

A model for Cpx system regulation is presented in Fig. 6. Under non-inducing conditions, CpxP is bound to the sensor-kinase CpxA inhibiting its kinase activity and preventing phosphorylation of the response regulator CpxR. When conditions exist that lead to the accumulation of misfolded and/or aggregated proteins in the periplasm, CpxP releases from CpxA and/or is degraded by the DegP protease (Thede et al., 2011). This then allows CpxA to autophosphorylate itself and then transfer the phosphoryl group to a conserved aspartate in the receiver domain of CpxR forming CpxR-P. CpxR-P acts as a transcriptional regulator acting to up-regulate or down-regulate the genes mentioned above (MacRitchie et al., 2008; Rowley et al., 2006). Thus, in contrast to the σ^E activation pathway (Fig. 2), which is a system that is "turned off" and needs to be "turned on", the Cpx system is "turned on" and needs to be "turned off" (by CpxP; Fig. 6).

As with the σ^E-regulon, the Cpx system also contributes to *S.* Typhimurium pathogenesis (Rowley et al., 2006). Cpx system appears to play a role in controlling the invasiveness of *S.* Typhimurium in a pH-dependent manner. CpxA, independent of CpxR, is needed for *hilA* (positive regulator of SPI-1 genes) expression at pH 6.0 but not pH 8.0 (Nakayama et al., 2003). In addition, a *cpxA* null mutant of *S.* Typhi was defective in adherence to, and invasion of, epithelial cells *in vitro* (Leclerc et al., 1998). Similarly, *cpxA** mutants (signal-blind constitutively active Cpx system) of *S.* Typhimuirum were also defective in adherence to, and invasion of, cells *in vitro*; but, exhibited wild type survival and growth within macrophages (Humphreys et al., 2004). It is not completely clear why a *cpxR* mutant does not behave in a similar way compared to *cpxA** or *cpxA* null mutants, but it is possible that under some conditions CpxA can phosphorylate a different response regulator (*e.g.*, OmpR).

2.4.4. Phage-shock protein response and envelope stress

The phage-shock-protein (Psp) response is another system induced by envelope stress (Becker et al., 2005; Darwin, 2005; MacRitchie et al., 2008; Rowley et al., 2006; Ruiz & Silhavy, 2005). The Psp resgulon is composed of the *pspABCDE* operon and two other genes; *pspF* (adjacent upstream and transcribed in the opposite orientation to *pspABCDE*) and *pspG*. PspA was the first protein identified as an *E. coli* protein up-regulated in response to infection with filamentous phage, in particular, increased expression and mislocalization of the phage-encoded secretin gene IV protein. It was later determined that other stresses, such as exposure to ethanol and hyperosmotic shock, could also up-regulate Psp regulon expression. Based upon the conditions (*e.g.*, defects in Sec-dependent secretion) and stresses (*e.g.*, hyperosmotic conditions and ethanol exposure) that can induce the Psp response, the inducing signal recognized by the Psp system is proposed to be a dissipation/loss of proton motive force (PMF) or membrane potential. As a result, the PSP response was classified as another envelope stress response system (Darwin, 2005; MacRitchie et al., 2008; Rowley et al., 2006).

The PspA protein was found to negatively regulate, while the PspB and PspC proteins act together to positively control, Psp regulon expression. The PspF protein functions as a σ^{54}-dependent transcriptional actvator of psp gene expression. The functions of PspD, PspE and PspG have not been determined (Darwin, 2005; MacRitchie et al., 2008; Rowley et al., 2006). Under non-inducing conditions, PspA binds to PspF preventing it from activating σ^{54}-dependent transcription of the psp genes. Inducing conditions (*e.g.*, mislocalization of secretins or conditions diminishing membrane potential or PMF) appear to be sensed by the inner membrane proteins PspB and/or PspC promoting their interaction. In this model, PspB/C interaction in turn somehow results in the preferential binding of PspA to PspB/C freeing PspF to activate σ^{54}-dependent transcription of the psp genes. As an added twist, PspA accumulation at the cytoplasmic surface of the inner membrane is thought to help maintain the PMF, which would ultimately remove the inducing signal, reducing PspB and PspC interaction releasing PspA into the cytoplasm allowing it to bind and inhibit PspF function (Darwin, 2005; MacRitchie et al., 2008; Rowley et al., 2006).

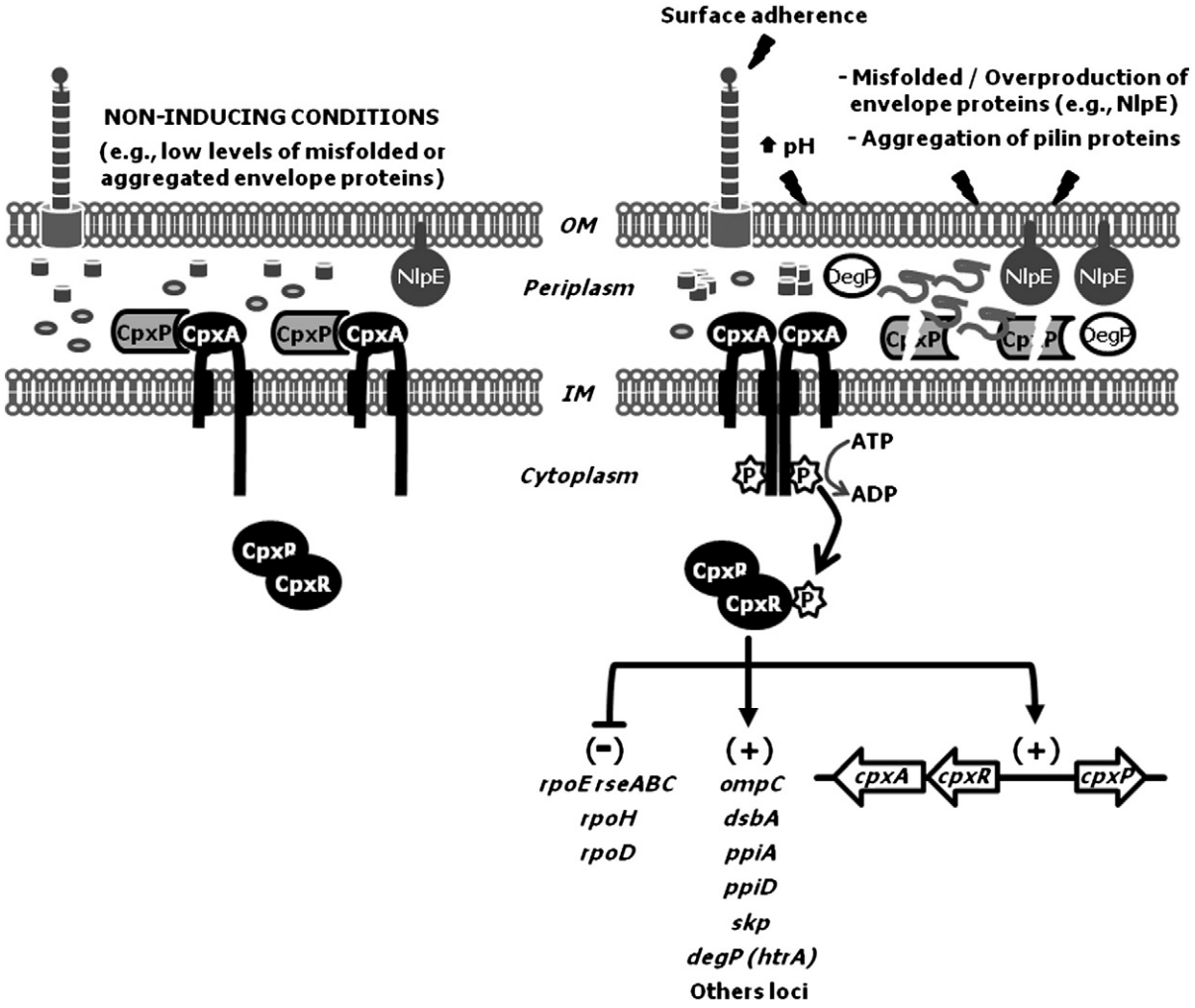

Fig. 6. Overview of the regulation CpxRA-CpxP pathway in *Salmonella* (MacRitchieet al., 2008; Rowleyet al., 2006). The CpxA sensor his kinase responds to stresses (*e.g.*, alkaline pH or overproduction of envelope proteins) that lead to the accumulation/aggregation or misfolding of envelope proteins. In the absence of these stimuli, CpxP binds to the periplasmic domain of CpxA inhibiting its kinase activity and ultimately the phosphorylation of CpxR. The accumulation or misfolding of proteins in the periplasm promotes CpxP degradation by the DegP (HtrA) protease. The release from CpxP inhibition allows CpxA to autophosphorylate itself and ultimately transfer the phosphoryl group to CpxR to form CpxR-P. CpxR-P then goes onto up-regulate and down-regulate the expression of a subset of genes/proteins including *cpxRA* and *cpxP*. The genes listed are examples of regulon members; see the corresponding text for additional genes and explanation. (+) indicates a positive effect (*e.g.*, up-regulation), while (−) indicates a negative effect (*e.g.*, down-regulation).

Early studies showed that the Psp system is induced by mislocalization of the filamentous phage secretin protein IV and mutations that cause the abnormal export of the proteins LamB and PhoE to the outer membrance. This suggested that the Psp system responds to the defective or saturated export of proteins through the Sec system (Darwin, 2005). However, it was later determined that this is not the situation, since studies with the proton ionophore carbonylcyanide chlorophenylhydrazone (CCCP) showed that dissipation of the PMF is what appears to be important in activating the Psp system (Kleerebezem et al., 1996; Weiner & Model, 1994). Becker,Bang, Crouch and Fang (2005) showed that, in S. Typhimurium, PspA levels increase in a *rpoE* null mutant. They also demonstrated that a mutant lacking both *rpoE* and *pspA* has reduced stationary-phase survival and a decreased PMF, compared to either an *rpoE* or *pspA* single mutant. This suggests that both the σ^E and Psp systems function in maintenance of the PMF and that PspA (or Psp response) can compensate for the lack of σ^E in this endeavor.

2.5. Antimicrobial peptide (AP) stress

Antimicrobial peptides (APs) are produced by bacteria, archaea, plants, and animals including humans. Soil bacteria (*e.g.*, *Paenibacillus polymyxa*) produce antimicrobial peptides – polymyxins and bacteriocins/lantibiotics – to kill competing microbes allowing them to better compete for nutrients (Choi, Park, et al., 2009; Pálffy et al., 2009). Higher organisms produce antimicrobial peptides as part of their innate defenses against microbial infections at the body surfaces. Various APs are produced in the skin and mucosal surfaces; and, are stored in the lysosomes of macrophages and neutrophils (Nizet, 2006; Pálffy et al., 2009; Peschel, 2002; Prost et al., 2007). Different types of APs have been identified that exhibit activity against a wide range of microbes including Gram-positive and Gram-negative bacteria, fungi, parasites and enveloped viruses. Some even target certain types of tumor cells (Pálffy et al., 2009). APs produced by eukaryotic organisms fall into two main families based upon their overall charge, cationic and non-cationic APs. The cationic AP family includes the (α- and β-) defensins, cathelicidins and thrombocidins. The non-cationic AP family members exhibit much lower antimicrobial activity with many being derived from other polypeptides/proteins of diverse functions (Pálffy et al., 2009).

Several models have been put forth for the mechanisms of action for some of the best studied cationic APs, defensins and other amphipathic cationic peptides. Basically, cationic APs are proposed to bind through their positively-charged regions to negatively-charged areas on microbial surface molecules (*e.g.* lipopolysaccharide or LPS of Gram-negative bacteria) aggregate and form channels or pores within the membrane. These pores allow for leakage of molecules (including the AP itself)

across the membrane and eventual cell death by osmotic lysis. The mechanism of pore formation is still unclear and may be different for different APs. Other mechanisms of killing have also been proposed based on findings that some APs (*e.g.*, cathelicidins) can inhibit DNA and/or protein synthesis (Boman et al., 1993; Gutsmann et al., 2001; Pálffy et al., 2009).

Resistance to the killing effects of APs depends on the surface structures the target organism produces, which the APs must traverse to reach the cytoplasmic membrane – the proposed primary target of toxicity for most APs (Nizet, 2006). Since salmonellae are Gram-negative bacteria, we will limit the discussion of AP resistance mechanisms to Gram-negative bacteria and in particular *S. enterica*. AP resistance mechanisms reported for *S.* Typhimurium (the best studied of the salmonellae) fall into two general schemes: (a) modification of cell surface components to reduce their negative-charge and, thus, binding to cationic AP molecules and (b) extracellular proteolytic degradation of certain APs by an outer membrane-associated protease.

2.5.1. AP resistance mediated by LPS modification

The former primarily involves several possible LPS modifications (Fig. 7) including the addition of 4-aminoarabinose (Ara4N) and/or phosphoenthanolamine (pEtN) to one or both phosphate groups in the lipid A moiety. This reduces its overall negative-charge at the outer membrane and, thus, its ability to bind to cationic APs. Ara4N addition requires the *pmrE* (*pmr* refers to a role in *p*oly*m*yxin *r*esistance) and *pmrHFIJKLM* operon (except for the *pmrM* gene product) gene products. The pEtN addition involves the *pmrC (eptA)* gene product (the first gene of the *pmrCAB* operon). Other LPS modifications include: (a) pEtN addition to the first heptose phosphate residue in the core polysaccharide, mediated by the *cptA* gene product and (b) dephosphorylation of the second core heptose phosphate by the *pmrG* gene product. All these genes exhibit positive control by the PmrAB (BasRS), and in some cases PhoPQ, two-component system (TCS). PmrA (BasR; response regulator) and PmrB (BasS; sensor-His-kinase) are also controlled indirectly by the PhoPQ TCS (Gunn et al., 2000; Ernst et al., 2001; Peschel, 2002; Nizet, 2006; Gunn, 2008; Fig. 4). Another mechanism of LPS modification is the alteration of the acylation status or character of the lipid A moiety. The addition of palmitate to the second position of the N-linked 3-hydroxymyristate on the proximal glucosamine of lipid A is catalyzed by the product of the PhoP-regulated *pagP* gene, while removal of the 3-hydroxymyristate (*i.e.*, deacylation) from the third position of the proximal glucosamine is mediated by the PhoP-regulated *pagL* gene product. In addition, LpxO (PagQ) mediates the formation of a S-2-hydroxymyristate on the distal glucosamine of the lipid A moiety. It is not clear how these changes in lipid A acylation status or character leads to AP resistance; but, *pagP* mutants do show increased outer membrane permeability to APs, suggesting that increasing palmitate content on lipid A affects the ability of some APs to form a pore or channel in the outer membrane (Ernst et al., 2001; Guo et al., 1998; Nizet, 2006).

2.5.2. AP resistance mediated by surface peptidases/proteases

Another general mechanism for AP resistance described in *S.* Typhimurium is the production of a surface-associated protease that degrades the AP before it can interact with the outer membrane. The *pgtE* gene encodes a surface protease that exhibits some specificity for α-helical APs, *e.g.* C18G and human cathelicidin LL-37 (Guina et al., 2000; Haiko et al., 2009). Guina et al. (2000) reported that PgtE degrades C18G indicating that this is how PgtE mediates resistance to these types of APs. PgtE is an orthologue of the *E. coli* OmpT protease and *Yersinia pestis* Pla protease. All three are members of the Omptin family of transmembrane aspartate proteases possessing highly conserved β-barrel fold motifs (Haiko et al., 2009). Although PhoPQ does not directly regulate *pgtE* transcription or PgtE export, it does indirectly control PgtE activity (Guina et al., 2000) and expression through SlyA, which is regulated by the PhoPQ TCS (Navarre et al., 2005). Interestingly, PgtE, and other Omptin family members, function seems to require interaction with "rough" LPS of the bacteria, *i.e.*, they are inhibited by long *O*-polysaccharide chains present on "smooth" LPS. The wzz_{st} (*a.k.a.*, *cld* or *rol*) gene product functions as an *O*-polysaccharide chain length determinant and is up-regulated by activation of the PmrAB and RcsC/RcsD/RcsB TCSs, which would lead to increased *O*-polysaccharide chain length (Delgado et al., 2006). We know that *S.* Typhimurium residing in vesicles within mouse macrophages (i) produce LPS with significantly shorter *O*-polysaccharide chain lengths and (ii) increase PgtE expression and function (Haiko et al., 2009); but, it is unclear how or if these two events are coordinately regulated.

2.5.3. Other mechanisms of AP resistance

Pilonieta et al. (2009) reported that two genes *ydeI (omdA)* and *ygiW* encoding for periplasmic oligosaccharide/oligonucleotide-binding (OB)-fold proteins are implicated in polymyxin B resistance in *S.* Typhimurium. YdeI and YgiW appear to interact with the outer membrane β-barrel porin proteins OmpD (NmpC) and OmpF, respectively. This interaction is necessary for their roles in polymyxin B resistance; and, at least for, YdeI-OmpD interaction is required for resistance to cathelicidin APs. The *ydeI* gene is regulated by three separate TCS, RcsC/RcsD/RcsB (for regulator of capsule synthesis), PhoPQ and PmrAB, which supports its role in AP resistance since all three TCS contribute to polymyxin B resistance (Erickson & Detweiler, 2006; Pilonieta et al., 2009). The significance of the interaction between YdeI-OmpD and YgiW-OmpF and how it contributes to AP resistance in this bacterium has yet to be determined.

2.5.4. PhoPQ-regulation of AP resistance

As discussed above, PhoPQ is a major regulatory system in acidic pH and AP resistance as well as macrophage survival and *Salmonella* virulence in mice (Ernst et al., 2001; Perez et al., 2009; Prost & Miller, 2008; Prost et al., 2007). The scheme for PhoPQ sensing and responding to acidic pH (*i.e.*, H^+ ions) was described above (Fig. 4); but brief further discussion of the signals that PhoQ recognizes is warranted here. PhoQ, the sensor-His-kinase component of this TCS, is an inner membrane protein possessing a sensor domain exposed to the periplasmic compartment of the cell and a cytoplasmic domain containing a HAMP linker domain, an autophosphorylation His box site and C-terminal ATP binding kinase/phosphatase domain. PhoQ is found as a dimer within the inner membrane and is capable of sensing certain periplasmic environmental signals to stimulate *trans*-autophosphorylation (using ATP) at conserved histidine residues within each monomer (Prost & Miller, 2008). This phosphoryl group is ultimately transferred to an aspartate residue on the cognate response regulator PhoP to form phosphorylated-PhoP (PhoP-P). PhoP-P functions as a transcriptional regulator to activate (*pag* genes; *P*hoP-*a*ctivated *g*ene) or repress (*prg*; *P*hoP-*r*epressed *g*ene) about 3% of the genes on the *S.* Typhimurium chromosome (Kato & Groisman, 2008; Prost & Miller, 2008; Fig. 4). PhoQ senses several environmental signals including divalent cations/metal ions (*e.g.*, Mg^{2+}, Ca^{2+} and Mn^{2+}), acidic pH and cationic AP. PhoQ is kept in a "repressed" state primarily by high concentrations of Mg^{2+} ions (typical concentrations it sees *in vivo*); the divalent metal ions bind to anionic regions in the PhoQ sensor domain and negatively-charged groups in the membrane resulting in divalent cation bridges that keep PhoQ in a repressed state. This allows PhoQ to respond to precipitous drops in Mg^{2+} ion concentrations leading to PhoP-P formation. Under these conditions, PhoP-P induces the expression of Mg^{2+} transporters that play a role in Mg^{2+} homeostasis (Choi, Groisman, & Shin, 2009; Kato & Groisman, 2008; Perez et al., 2009; Prost & Miller, 2008). Interestingly, acid pH activation of PhoPQ results in induction of the *feoB* gene involved in Fe^{2+} transport into the cell; providing another link between the PhoPQ TCS controlled metal ion homeostasis and PhoPQ-regulated stress responses (Choi, Groisman, & Shin, 2009). PhoQ is not only capable of binding to Mg^{2+} and H^+ ions; it is also able to bind to cationic APs (Bader et al., 2005). In fact, cationic APs, and H^+ ions, are the most likely environmental signals activating the PhoPQ system *in vivo*.

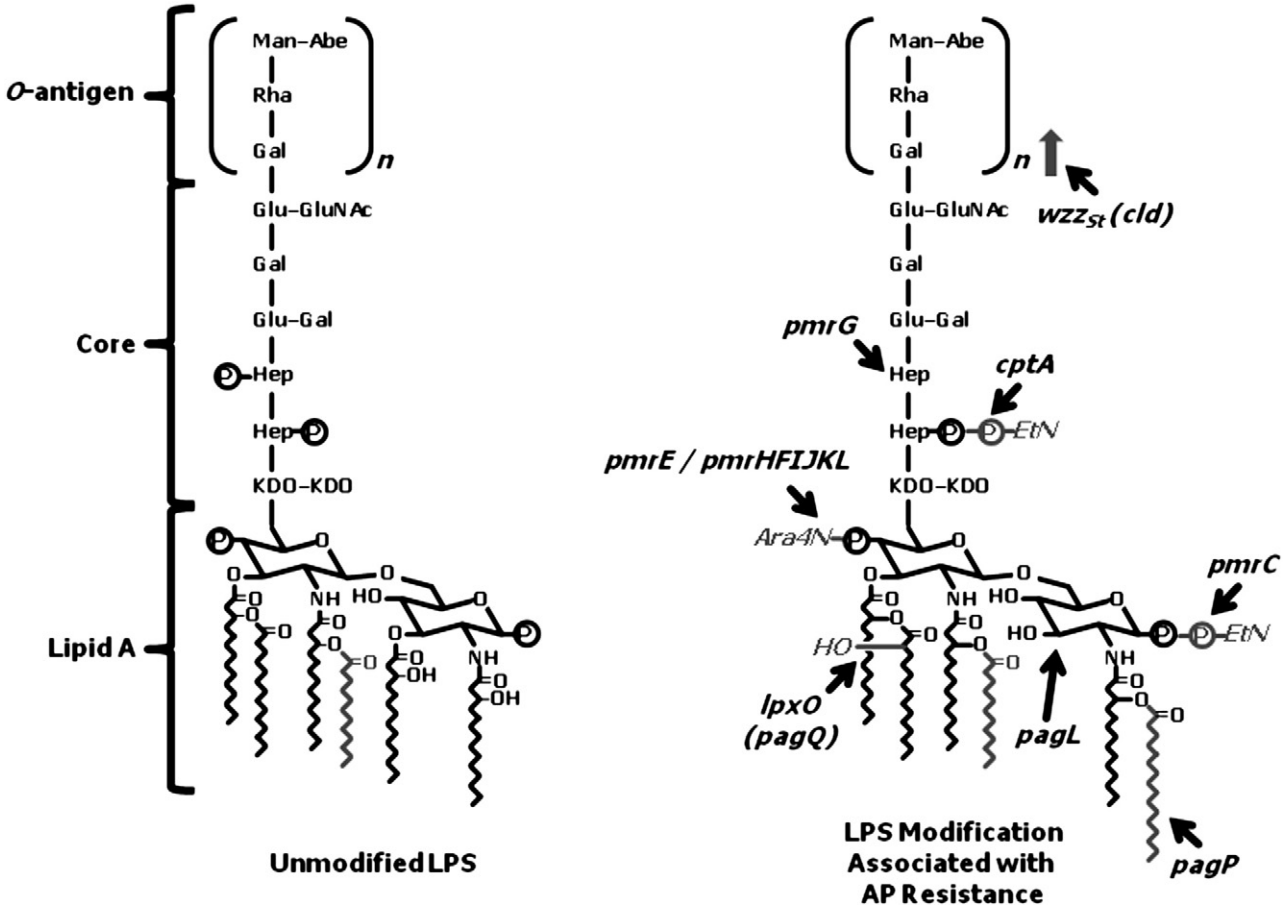

Fig. 7. Lipopolysaccharide (LPS) modifications contributing to antimicrobial peptide (AP) resistance in *Salmonella* (Gunn, 2008). Many of the modifications to the LPS function to reduce the negative charge of the LPS molecule, particularly near the OM surface, in order to lessen its interaction with positively charged molecules, cationic APs. The *pmrG, pmrC (eptA), pmrE, pmrHFIJKL, cptA* and wzz_{St} genes are all up-regulated by PmrA-P. Modifications that alter the acylation status or character of the lipid A moiety likely affect membrane permeability to APs and are all up-regulated by PhoP-R (*i.e., pagL, pagP,* and *lpxO (pagQ)*). The wzz_{St}*(cld)* gene product controls *O*-antigen chain length. See the corresponding text for additional explanations.

Subinhibitory levels of cationic APs will activate PhoPQ even in the presence of high physiological concentrations of Mg^{2+} ions (Bader et al., 2005). Cationic APs bind to the same sites in the PhoQ dimer as divalent metal ions but with much greater affinity. Thus, cationic APs can effectively disrupt the divalent cation bridges that keep PhoQ in a repressed state. This activates the PhoPQ system and directly, or indirectly through PmrAB, induces the expression of genes involved in AP resistance and virulence (Kato & Groisman, 2008; Prost & Miller, 2008).

2.5.5. PmrAB (BasRS)-regulation of AP resistance

Another TCS that contributes to AP resistance is PmrAB. PmrB has an analogous function to PhoQ in this system sensing such environmental cues as high Fe^{3+} or Al^{3+} ions, acidic pH (*e.g.*, pH 5.5) and vanadate (Gunn, 2008; Wosten et al., 2000). Like PhoQ, PmrB is an inner membrane protein possessing a periplasmic sensor domain and cytoplasmic domains necessary for autophosphorylation of a histidine residue and subsequent phosphorylation of an aspartic acid in PmrA to form phosphorylated-PmrA (PmrA-P). PmrA-P functions as a response regulator controlling the expression of *pmr* genes, described above (Fig. 4). The periplasmic sensor domain of PmrB possesses two putative iron-binding sites (ExxE) but the Fe^{3+} concentrations needed to activate PmrB are likely irrelevant *in vivo*; but, in soil iron and aluminum are among the most abundant metals, suggesting that iron and/or aluminum activation of PmrAB may be important for life outside the host as well. The PmrAB TCS is also activated directly and indirectly, via PhoPQ activation, by acidic pH. *S.* Typhimurium grown at pH 5.8 is much more resistant to polymyxin B than cells grown at pH 7.7 (Gunn, 2008). As with PhoQ, the exact mechanism of how PmrB senses H^+ ions is not clear. PmrAB is also indirectly activated through a PhoPQ-dependent mechanism. PhoPQ positively controls the expression of *pmrD* whose gene product (9.6 kDa PmrD protein) regulates PmrA activity post-transcriptionally by binding to PmrA-P and stabilizing it. PmrA-P in turn feedback controls this process by repressing *pmrD* transcription (Fig. 4). This PmrD-mediated PmrA stabilization mechanism seems to be specific to *Salmonella* and allows for the activation of PmrAB and PhoPQ to be connected (Gunn, 2008). This PmrD-mediated mechanism may be the most important means of PmrAB activation *in vivo* since the transcription from PmrA-regulated promoters is greatly reduced in a *phoP* null mutant (Merighi et al., 2005).

2.5.6. Cell envelope stress responses and AP resistance

As mentioned previously, induction of the SSR in *S.* Typhimurium generates a cross-resistance to polymyxin B and other APs, and that this AP resistance requires the *rpoE* gene product σ^E (McLeod & Spector, 1996; Kenyon et al., 2002; Spector, unpublished data). Crouch et al. (2005) subsequently reported that σ^E is required for resistance to bactericidal/permeability-increasing protein (BPI)-derived peptide P2 and the mouse α-defensin Crp-4. These researchers also demonstrated that exposing stationary-phase cells to sub-inhibitory levels of either P2 or Crp-4 increased *rpoE* mRNA levels when compared to untreated stationary-phase cells. This supports the idea that APs generate either distinct or overlapping (*e.g.*, heat or

C-starvation generated) extracytoplasmic stress signals that activate the σ^E regulon (Crouch et al., 2005).

In a recent report, the CpxRA TCS is shown to play a role in resistance to certain APs (Weatherspoon-Griffin et al., 2011). These researchers demonstrated that CpxRA activation, resulting from NlpE overexpression, promotes resistance to protamine. This resistance is found to result from its up-regulation of two peptidoglycan (*N*-acetylmuramoyl-L-alanine) amidases — encoded by the *amiA* and *amiC* genes. These two amidases increased resistance not only to protamine but also the α-helical APs magainin 2 and melittin (Weatherspoon-Griffin et al., 2011). This further supports the idea that structures within the cell envelope (peptidoglycan and outer membrane) can play a role in resistance to different APs.

2.5.7. Other genes involved in AP resistance

Detweiler et al. (2003) also showed that *rcsC* (encodes a sensor-His-kinase), *virK* (encoding a putative *Shigella* VirK homologue) and *somA* (encoding a putative VirK homologue) are all involved in polymyxin B resistance. Interestingly, *virK* and *somA* are PhoP-regulated but *rcsC* is not; although *rcsC* is OmpR-regulated. These researchers proposed that these loci, like others described above, contribute to outer membrane remodeling in response to environmental cues.

2.6. Bile resistance and multidrug resistance

Foodborne pathogens such as *S. enterica* serovars not only must survive the effects of the stomach acidity and antimicrobial peptides secreted by the intestinal mucosa but also the antimicrobial action of bile. Bile is released into the distal small intestine from the gallbladder following ingestion of a meal, particularly one high in fat. It is composed of fats and phospholipids, such as cholesterol and phosphatidylcholine, bile salts and a relatively small number of proteins (*e.g.*, immunoglobulin). The antimicrobial activity of bile is primarily the result of the bile salts – produced from cholesterol by the liver and secreted into the bile. In general, enteric bacteria such as *Salmonella* are resistant to bile salts – a characteristic that has been exploited to selectively culture enteric bacteria while excluding other microbes. Nonetheless, the relatively high concentration of bile in the distal small intestine generally keeps colonization of these sites by normal microbial flora to a minimum. Thus, high concentrations of bile are inhibitory for even the more resistant enteric bacteria (Gunn, 2000; Merritt & Donaldson, 2009).

How bile salts mediate their antimicrobial activities is still not fully understood; but the effects of certain bile salts can be deduced based on mutations that result in bile sensitivity. Although, bile salts are surface-active agents with detergent-like action this likely does not account for their entire antimicrobial activity. Several mutations that make cells sensitive to different bile salts are in genes encoding factors involved in DNA repair pathways as well as in systems that actively pump bile salts out of the cytoplasm (*e.g.*, multidrug efflux systems). This indicates that DNA and/or some other cytoplasmic molecule(s) is the ultimate target(s) for the antimicrobial action of some bile salts (Merritt & Donaldson, 2009).

Van Velkinburgh and Gunn (1999) showed that *S.* Typhimurium grown in the presence of a sub-lethal concentration of bile become resistant to extremely high concentrations of bile (24–30%). This adaptation occurred preferentially in exponentially growing cells compared to stationary-phase cells; and required a concentration of bile (15%) that was barely sub-lethal. The researchers also showed that *Salmonella* exposed to bile or deoxycholate alone exhibited altered patterns of protein expression compared to untreated cells. Interestingly, Van Velkinburgh and Gunn (1999) also found differences between the proteins expressed in cells exposed to bile compared to those exposed to the bile salt deoxycholate alone.

As mentioned above, *Salmonella* and other Gram-negative bacteria are generally resistant to bile. However, some lipophilic or uncharged forms of bile salts are able to cross through the outer membrane or enter through porins, *i.e.*, OmpF. As a consequence, three general schemes for bile resistance are observed in *Salmonella* and other Gram-negative bacteria: (a) the production of a long *O*-Ag polysaccharide chain (*i.e.*, smooth LPS; discussed earlier), but not the PmrAB or PhoPQ-dependent LPS modifications, provides an effective barrier to limit access of bile salts to the outer membrane; (b) the synthesis of efflux systems (*e.g.*, multidrug efflux systems) that act with certain Tol proteins to actively pump the bile acid out of the cell; and (c) the production of DNA repair enzyme pathways that are part of the SOS response (Merritt & Donaldson, 2009).

2.6.1. Multidrug efflux systems and bile resistance

One of the best-characterized mechanisms for bile resistance involves (multidrug) efflux systems that pump bile salts out of the cell's cytoplasm. Different efflux systems function in resistance to a variety of potentially toxic compounds including: bile salts, organic solvents, antibiotics, and oxidizing agents (Nishino et al., 2006, 2009).

The *acrAB* (for *acr*idine-sensitivity) encoded multidrug efflux system is one of the best-studied systems. It is required for resistance to bile salts and lipophilic antibiotics (*e.g.*, erythromycin) as well as several dyes, detergents and solvents (Gunn, 2000). AcrAB works in conjunction with TolC to "pump" bile salts, antibiotics, *etc.* out of the cell. In *Salmonella*, *acrAB* is induced by bile as well as indole and *E. coli* "conditioned" medium (presumably because it contains indole produced from tryptophan). The induction of *acrAB* under these conditions is dependent on the RamA protein but not Rob, MarA, AcrR, SoxS or SdiA, as in *E. coli* (does not possess *ramA*). The *ramA* (*r*esistance *a*ntibiotic *m*ultiple) gene product is proposed to be the major regulator of *acrAB* expression in *Salmonella* (Nikaido et al., 2008; Nishino et al., 2009). Abouzeed et al. (2008) further identified a gene *ramR*, upstream of *ramA*, that when mutated lead to increased expression of *ramA* and the AcrAB multidrug efflux pump, producing a multidrug resistance phenotype. Therefore, RamR is proposed to be a local repressor of *ramA* expression. Although, *ramA* gene expression is not induced by bile (in contrast to indole which does induce *ramA* expression), a bile component does bind to RamA and, in doing so, is proposed to act as an inducer to increase RamA's function as an activator of *acrAB* expression (Nishino et al., 2009). RamA is also an activator of *acrEF* and *tolC* expression. Furthermore, RamA appears to regulate the expression of multiple *Salmonella* pathogenicity island (SPI-1 and 2) genes affecting macrophage survival and virulence in the BALB/c mouse model (Bailey et al., 2010).

Prouty, Brodsky, Falkow, and Gunn (2004) showed that the *marRAB* (*m*ultiple *a*ntibiotic *r*esistance), like the *acrAB*, operon is activated by bile salts. The MarR protein is a transcriptional repressor of the *marRAB* operon and MarA is a transcriptional activator of several antibiotic-resistance genes including *acrAB*. However, as mentioned above, MarA is not required for increased expression of AcrAB in response to bile (Nikaido et al., 2008). MarA does up-regulate the expression of *micF* (an antisense RNA that down-regulates *ompF* mRNA translation) which would lead to reduced OmpF expression (Alekshun & Levy, 1997). Decreased OmpF porin could reduce the entry of bile salts across the outer membrane, which might contribute to the bile sensitivity phenotype of *marRAB* mutants in the presence of bile in *S.* Typhimurium (Merritt & Donaldson, 2009). Prieto et al. (2009) identified a null mutation, in the *asmA* gene, that suppresses the bile-sensitivity phenotypes of several mutants and enhances bile resistance in wild type strains. Lack of AsmA (a putative outer membrane protein) increases *marRAB* operon transcription, which could account for the increased bile resistance observed. The mechanism by which the lack of AsmA leads to *marRAB* activation is not clear. Those member(s) of the MarA regulon involved in the increased bile resistance in an *asmA* mutant is likewise unknown. It has been proposed that failure to produce a functional AsmA protein somehow causes a reorganization of the outer membrane that either directly or indirectly produces a signal that up-regulates *marRAB* transcription (Prieto et al., 2009). However,

the idea that lack of AsmA somehow disrupts OmpF assembly and therefore reduces bile entry into the periplasm was discounted based on findings in *E. coli* that the *asmA* null mutation did not affect assembly of OMPs (Misra & Miao, 1995); but this has not been confirmed in *Salmonella*. In addition, AsmA does not appear to regulate *acrAB* transcription suggesting that increased bile resistance is due to one or more of the other MarA regulon members (Prieto et al., 2009). The lack of an effect on *acrAB* transcription supports the finding that *acrAB* transcription is more RamA-dependent than MarA-dependent in *S.* Typhimurium (Nikaido et al., 2008). Interestingly, an *asmA* null mutant is defective in epithelial cell invasion *in vitro* and attenuated in the oral, but not intraperitonial, mouse virulence model (Prieto et al., 2009) possibly supporting a role in organization or biogenesis of outer membrane structures.

The BaeSR two-component system has also been shown to play a role in the bile resistance of *S.* Typhimurium. The BaeSR system is classified as an envelope stress response in *E. coli* because of initial studies showing some overlap with the Cpx activation and the Cpx regulon, both regulate the *spy* gene (MacRitchie et al., 2008). It is included here because of some of the loci it is found to up-regulate. In this system, BaeS functions as a sensor his-kinase embedded in the inner membrane and BaeR is its cognate cytoplasmic response regulator. Nishino et al. (2007) showed that overexpression of plasmid-borne *baeR* resulted in increased resistance to deoxycholate and other compounds including β-lactams, novobiocin, copper, and zinc. These phenotypes were determined to be the result of BaeR-P mediated induction of *acrD* and *mdtABC*, both encoding multidrug export systems (Nishino et al., 2005; Nishino et al., 2007). BaeR-P appears to autoregulate its own expression as part of the *mdtABCD-baeSR* operon as well. In addition, genomic analysis of gene expression in response to *baeR* overexpression indicates that BaeR is likely to control the expression of several more genes other than *mdtABSD-baeSR*, *acrD* and *spy* (Nishino et al., 2005). Thus, the evidence reported thus far, indicates that the BaeSR system is an envelope stress response (ESR) system that is responsible for regulating the expression of efflux pumps in response to specific compounds that can be toxic to the cell (*e.g.*, indole, certain antimicrobial agents and bile salts). However, the fact that spheroplast formation and overproduction of pilin protein PapG can also induce the BaeSR system suggests that it may also be involved in combating the effects of more general envelope stress (Leblanc et al., 2011; MacRitchie et al., 2008; Raffa & Raivio, 2002; Rowley et al., 2006).

Interestingly, activation of the SoxRS regulon confers resistance to various antimicrobial drugs as well. This may be related to the exclusion or removal of "foreign" molecules (*i.e.*, xenobiotics). This is supported by the fact that many of the promoters recognized by SoxS are also recognized by the global regulatory proteins MarA and Rob involved in multiple antibiotic resistances (Storz & Zheng, 2000).

Prouty, Van Velkinburgh, and Gunn (2002), using Mu*d*J-*lac* transposon mutagenesis in a PhoP-constitutive (PhoP^c) mutant background, identified three insertions that lead to bile sensitivity. All three insertions were ultimately found to be in or near the putative *orf1(ybgC)-tolQRA* operon; specifically in *tolQ, orf1* and an upstream putative gene *orfX* (*ybgE*; not previously known to be associated with Tol function). These three loci are co-transcribed indicating that *orfX* is a member of the operon, *orfX-orf1-tolQRA*. Additionally, *orfX* or *ybgE* is annotated as being a member of the *cyd* (cytochrome d terminal oxidase) operon (*cydAB-ybgTE*); but it is not known whether *orf1-tolQRA* are also part of a larger operon beginning at *cydA*. None of these fusions exhibited regulation by bile or known regulators of either bile resistance or the *E. coli tolQRA* genes, *i.e.* PhoPQ or RcsCB TCSs (Prouty, Van Velkinburgh, & Gunn, 2002). In addition to increased bile sensitivity, *tolQRA* mutants show increased sensitivity to certain antibiotics and detergents. However, they are resistant to colicin A and infection with filamentous bacteriophages. These phenotypes are consistent with the function of the Tol-Pal system in maintaining outer membrane integrity, LPS production, colicin A transport and efflux system function (Paterson et al., 2009).

2.6.2. DNA repair mechanisms and bile resistance

Bile salts cause DNA damage and induce the SOS response (in a RecA-dependent manner) in *S. enterica*. Numerous mutations in genes associated with DNA repair mechanisms result in bile sensitivity (Merritt & Donaldson, 2009). Among these are mutations in genes encoding (i) DNA adenine methyltransferase (Dam), (ii) mismatch repair proteins MutH, MutL and MutS, (iii) base-excision repair proteins, exonuclease III (Xth) and endonuclease IV (Nfo), (v) SOS response-associated translesion DNA polymerase (DinB) and (iv) recombination (repair) proteins RecBCD and RecA.

Bile salts are found to increase the frequency of GC → AT transition mutations. The bile sensitivity phenotypes of DNA repair mutants suggest that bile salts can also increase the frequency of frameshift mutations (*e.g.*, small insertion or deletion mutations) and chromosomal rearrangements. Bile salts also induce the expression of several OxyR and SoxRS regulon members suggesting that bile salts can cause DNA damage in a similar manner as oxidative stress (Merritt & Donaldson, 2009; Prieto et al., 2006). Prieto et al. (2006) presented a model in which bile salts generate a DNA lesion requiring Dam-directed mismatch repair and/or base-excision repair mechanisms for repair. These processes would generate single-stranded DNA regions/DNA strand breaks that induce the SOS response. Additionally, the SOS response can be induced if the resulting DNA lesion(s) blocks DNA replication. This block can be overcome by translesion DNA replication mediated by the SOS response-associated DNA polymerase DinB or via RecBCD-mediated recombinational repair (Merritt & Donaldson, 2009; Prieto et al., 2006).

López-Garrido et al. (2010) recently reported that a mutation in the gene upstream of *dam* within the *aroKB-damX-dam-rpe-gph* operon in *S.* Typhimurium resulted in bile sensitivity. Non-polar *damX* mutations and complementation with the wild type *damX* gene indicates that bile sensitivity is due to loss of DamX and not polar effects on Dam expression. DamX is a predicted 46 kDa inner membrane protein that runs much larger on SDS-PAGE and is expressed in both growing and stationary-phase cells. Interestingly, the bile sensitivity phenotype of a *damX* mutant is suppressed in an *asmA* null mutant; although, it is not clear why (López-Garrido et al., 2010). Not surprisingly, many of the genes involved in DNA repair and bile resistance are also important for virulence of *S. enterica*; further supporting the importance of bile resistance and, in particular, the ability to repair DNA in the virulence potential of *Salmonella*.

2.6.3. Additional genetic mutations/genes associated with bile resistance

As eluded to above, the PhoPQ TCS is implicated in bile resistance based upon the phenotypes exhibited by PhoP constitutive (PhoP^c) and *phoP* null mutants. A PhoP^c mutant is able to survive extended periods at bile concentrations of greater than 60%; while a *phoP* null mutant is significantly more bile sensitive than a wild type strain. Moreover, PhoPQ-regulated bile resistance specifically targets deoxycholate and conjugated forms of chenodeoxycholate. Interestingly, PhoPQ does not sense bile or its components and, therefore, bile does not induce the PhoPQ-regulon. However, (the PhoP-repressed genes) *prgC* and *prgH* are repressed by bile in a PhoPQ-independent manner (Van Velkinburgh & Gunn, 1999). In a later study, Gunn et al. (Prouty, Brodsky, Manos, et al., 2004) reported that *pagC* is down-regulated in the presence of bile in a PhoP-independent manner requiring a 97-bp region in the untranslated leader sequence of *pagC* for bile-mediated repression. Surprisingly, none of the known PhoP-activated or -repressed genes tested appeared to play a role in bile resistance. A possible explanation for *phoP* mutant phenotypes is that the PhoPQ-regulon and the bile-induced regulon share common members, which are required for bile resistance (Van Velkinburgh & Gunn, 1999).

Prouty, Brodsky, Manos, et al. (2004) identified the YciF protein (a putative ferritin-like iron/metal binding protein) as a bile-regulated protein and the *yciGFE-katN* operon as bile-inducible. Interestingly,

this operon was previously determined to be σ^S-regulated, but its responsiveness to bile is σ^S-independent.

2.6.4. Response to bile and biofilm formation

Gunn et al. (Crawford et al., 2008; Prouty, Schwesinger, & Gunn, 2002b) reported that bile can promote biofilm formation by *Salmonella* serovars on gallstones. Gallstone development and the ability to form a biofilm on them are important to the colonization of the gall bladder and the development of a carrier state during *Salmonella* infection. The ability to form a biofilm on gallstones is independent of the ability to produce colonic acid or cellulose, suggesting that production of another exopolysaccharide is necessary for gallstone biofilm formation. Using a cholesterol-coated polypropylene tube assay, these researchers (Crawford et al., 2008) showed that *O*-antigen capsule production (requiring the *yihU-yshS* and *yihV-yihW* operons for assembly and extracellular translocation) is necessary for biofilm formation on cholesterol-coated surfaces but not glass or plastic surfaces for *Salmonella* serovars Typhimurium, Typhi and Enteriditis. The presence of bile also induces the *O*-antigen capsule genes independent of *agfD* (Crawford et al., 2008). Thus, the responses of salmonellae to bile are not only important for virulence but also the establishment of chronic infections or carrier states in the host.

2.7. Osmotic stress

Serovars of *S. enterica* are able to adapt rapidly to changes in osmotic pressure as they cycle through various host and non-host environments (Foster & Spector, 1995; Winfield & Groisman, 2003). Whether in response to an osmotic upshift leading to a hypertonic environment or an osmotic downshift leading to a hypotonic environment, bacterial cells attempt to maintain the appropriate turgor pressure via a corresponding increase or decrease in the concentration of solutes in the cytosol and/or periplasm (Bremer & Krämer, 2000; Poolman et al., 2002; Wood, 2006; Wood et al., 2001). The genetic and physiological responses to osmotic stress often involve an immediate countermeasure to quickly balance osmotic pressure, which is then replaced by a more long-term readjustment to the osmotic conditions in the surrounding environment. These defenses against osmotic stress are essential for the survival and continued growth of the bacteria.

2.7.1. Responses to hyperosmotic stress

An osmotic upshift results in a net movement of water molecules across the inner membrane and the loss of water from the cytoplasm. Water passes either directly through the phospholipid bilayer or through specific protein channels such as the AqpZ aquaporin (Calamita et al., 1995; Delamarche et al., 1999). Within only a few minutes, transcription of the *kdpABC* operon, encoding a high-affinity K^+ transport system, is induced. This results in a rapid accumulation of intracellular K^+ due to the combined actions of both the Kdp system and the constitutive, low-affinity Trk transport system (Balaji et al., 2005; Bremer & Krämer, 2000). Postassium transport is followed by an increase in glutamate, which serves as a counterion (Botsford et al., 1994; Bremer & Krämer, 2000). Whether a loss of turgor pressure or an increase in intracellular ionic strength is repsonsible for triggering this initial response to osmotic stress is still a matter of debate (Balaji et al., 2005; Poolman et al., 2002; Wood, 2006; Wood, 2007).

The increase in intracellular K^+ is a temporary solution that is promptly replaced by an accumulation of organic compounds known as compatible solutes. These more physiologically friendly osmoprotectants include proline, glycine betaine, ectoine, or trehalose and are either transported into the cell from the extracellular environment or are synthesized *de novo* (Empadinhas & da Costa, 2008; Roeßler & Müller, 2001). In *Salmonella* and *E. coli*, the genetic loci *proU* and *proP*, encoding an ABC transporter complex and a H^+ symporter, respectively, are both upregulated in response to hyperosmotic stress (Balaji et al., 2005; Bremer & Krämer, 2000; Wood, 2007). The ProP and ProU membrane transport systems are capable of transporting a number of different compatible solutes into the cell including glycine betaine, proline betaine, and proline. Choline, a precursor for the biosynthesis of glycine betaine, is transported across the inner membrane by the BetT protein in *E. coli* (Bremer & Krämer, 2000; Wood, 2007). In addition, the outer membrane protein OmpC, which is upregulated in response to hyperosmotic conditions via the EnvZ/OmpR two-component regulatory system (Fig. 5), may provide a channel for certain compatible solutes such as glycine betaine to enter the periplasm (Faatz et al., 1988). Interestingly, the EnvZ/OmpR phosphorelay system is required for full virulence in *S.* Typhimurium (Foster & Spector, 1995).

If no exogenous compatible solutes are available for transport, or if the supply of these compounds is not adequate to balance osmotic forces, *Salmonella* serovars synthesize the non-reducing, disaccharide trehalose as their main osmoprotectant. Trehalose biosynthesis involves the enzymes trehalose-6-phosphate phosphatase and trehalose-6-phosphate synthase encoded by the genes *otsB* and *otsA*, respectively (Kaasen et al., 1992; Strøm & Kaasen, 1993). The *otsBA* operon is controlled by the general stress response sigma factor σ^S, which is known to also regulate other genes involved in osmotolerance (Du et al., 2011; Hengge, 2008; Ibanez-Ruiz et al., 2000; Kaasen et al., 1992). In fact, the RNAPσ^S holoenzyme complex appears to overcome the transcriptional repression of osmoregulated genes mediated by the DNA-binding protein H-NS (Atlung & Ingmer, 1997; Fang & Rimsky, 2008; Typas et al., 2007). Furthermore, the σ^S regulon is critical for the virulence of *S.* Typhimurium, again suggesting that responses to osmotic stress play a role in pathogenesis (Fang et al., 1992; Spector, 1998).

2.7.2. Responses to hypoosmotic stress

Salmonellae likely encounter low osmolarity conditions in non-host, freshwater environments as well as within specific microenvironments of host organisms. An osmotic downshift results in a net movement of water into the cell and an increase in turgor pressure. The peptidoglycan cell wall of Gram-negative bacteria functions to prevent the inner membrane from rupturing, but additional mechanisms are needed to releave osmotic pressure. Mechanosensitive channels, such as MscL, MscM, and YggB, located in the inner membrane of many Gram-negative bacteria sense membrane tension and mediate the release of compatible solutes, restoring osmotic balance (Booth & Louis, 1999; Kung et al., 2010; Levina et al., 1999; Morbach & Krämer, 2002; Poolman et al., 2002; Sukharev et al., 1997).

Another strategy employed by *S. enterica* to counteract the detrimental effects of hypoosmotic stress is the accumulation of osmoregulated periplasmic glucans (OPGs), also known as membrane-derived oligosaccharides (MDOs) (Bohin, 2000; Lee et al., 2009). The *opgGH* operon of *S.* Typhimurium is induced under hypotonic conidtions leading to the biosynthesis and transport of glucose polymers linked primarily by β-1,2 glycosidic bonds (Bhagwat et al., 2009). A *S.* Typhimurium *opgGH* mutant (deficient in OPG production) exhibits impaired growth, motility, and biofilm formation under low osmolarity conditions, as well as a decreased ability to colonize mouse organs and attenuated virulence in mice (Bhagwat et al., 2009; Liu et al., 2009). Therefore, the *Salmonella* strategies for adapting to hypoosmotic stress are likely to be important for survival in non-host environments, but also appear to play a critical role in pathogenesis.

2.8. Desiccation stress

Serovars of *S. enterica* possess a number of defenses against the harmful effects of desiccation. Water loss through evaporative drying or through matric water stress is an important factor affecting the survival and persistance of salmonellae and other bacteria on inanimate surfaces, on plant surfaces, in dried and low water-content food products, and in environmental habitats such as soil (Billi & Potts, 2002; Potts, 1994; Potts, 2001). While certain cell components of *Salmonella* may help to

slow the drying process and prevent complete desiccation, others appear to maintain cell viability by protecting membranes and proteins in a desiccated state. Comparitively little is known about the genetic and physiological responses to desiccation stress in *Salmonella*, but it is already clear that there is significant overlap with other stress response networks which are likely to be simultaneously induced during desiccation including those reacting against osmotic, thermal, and oxidative stress (Gruzdev et al., 2011).

2.8.1. Extracellular defenses against desiccation

Bacterial glycocalyces are known to have a variety of functions including a role in protecting bacteria against complete desiccation (Ophir & Gutnick, 1994; Tamaru et al., 2005). Normally composed of exopolysaccharides and associated proteins, glycocalyx surface layers form a gel-like extracellular matrix that often holds significant amounts of bound water. This water is lost slowly to evaporative and matric forces that decrease the water activity of the surrounding environment (Ophir & Gutnick, 1994; Potts, 1994). Other colloidal surface structures may also be important in slowing the drying process. For *S.* Typhimurium, extracellular cellulose and the thin aggregative fimbriae known as curli have proven to be major factors in desiccation resistance and survival (Barnhart & Chapman, 2006; White et al., 2006). The regulatory protein CsgD controls the biosynthesis of both extracellular cellulose and curli and is required for establishment of desiccation-resistant *Salmonella* biofilms (Gerstel & Römling, 2003; Jain & Chen, 2007; Römling, 2005). Colony morphology variants produce differing combinations of curli and cellulose (Römling, 2005; White & Surette, 2006). The rdar colony morphotype produces both curli and cellulose, bdar colonies produce curli only, and pdar colonies produce cellulose only. The rdar (red, dry, and rough) morphotype, possessing both curli and cellulose, is the most resistant to desiccation stress (Vestby et al., 2009; White et al., 2006). Additionally, the O-antigen polysaccharide chain of LPS appears to play an important role in the desiccation resistance of *S.* Typhimurium (Garmiri et al., 2008).

2.8.2. Intracellular defenses against desiccation

The disaccharide trehalose not only acts as a compatible solute but also appears to aid in maintaining the structure and function of proteins and membrane lipids during drying (Crowe et al., 1992; Elbein et al., 2003; Furuki et al., 2009; Potts, 1994). In fact, trehalose may essentially replace water under conditions of extreme desiccation, preventing denaturation of proteins and stabilizing membrane phospholipids (Crowe et al., 1992). Interestingly, trehalose also protects *Salmonella* against other forms of stress, which might be associated with desiccation such as thermal stress and oxidative stress (Cánovas et al., 2001; Crowe et al., 2001; França et al., 2007; Howells et al., 2002). As mentioned above, the *otsBA* operon responsible for trehalose biosynthesis is regulated by σ^S, which also controls a wide range of physiological functions necessary for resistance to osmotic stress, temperature stress, and oxidative stress, again illustrating the degree of overlap between various stress response networks of *Salmonella* (Elbein et al., 2003; Furuki et al., 2009; Ibanez-Ruiz et al., 2000; McMeechan et al., 2007).

2.9. Iron stress

Iron (Fe^{3+} or Fe(III)) is an essential nutrient for bacterial metabolism and growth. Iron serves as an important cofactor for proteins involved in a number of physiological processes ranging from cellular respiration, to DNA replication and repair, to the regulation of gene expression (Benjamin et al., 2010; Skaar, 2010). Iron is an abundant element, but ironically, free Fe^{3+} is seldom available to bacteria because of its insolubility or because it is sequestered by high affinity, iron-binding proteins such as transferrin and lactoferrin within vertebrate hosts (Hantke & Braun, 2000; Skaar, 2010). In fact, under most conditions encountered by salmonellae, the concentration of free Fe^{3+} is typically far below the concentration required for growth (Foster & Spector, 1995; Hantke & Braun, 2000).

2.9.1. Siderophore-mediated iron aquisition

Not surprisingly, most pathogenic bacteria have evolved strategies to circumvent host defenses in the battle for iron. For example, *Neisseria* and *Moraxella* species produce transferrin and lactoferrin receptors allowing for the direct uptake of bound iron from these proteins (Beddek & Schryvers, 2010). Other Gram-negative bacteria such as *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* express receptors for the uptake of heme, hemoproteins, or hemophores (Cope et al., 1995; Ghigo et al., 1997; Lettofe et al., 1998). An alternative strategy employed by *Escherichia coli* and *Salmonella enterica* involves secretion of low-molecular-weight, iron-chelating compounds known as siderophores (Chu et al., 2010; Wandersman & Delepelaire, 2004). Siderophores have such a high affinity for iron that they can directly remove it from host iron-binding proteins (Fischbach, Lin, Liu & Walsh, 2006; Ratledge, 2007). The classic catecholate siderophore enterobactin is synthesized by *S.* Typhimurium (Pollack & Neilands, 1970; Raymond et al., 2003). In *Salmonella*, ferric-enterobactin is transported into the cell through either FepA or IroN outer membrane proteins (OMPs) by an active transport mechanism involving the energy-transducing Ton system (Bäumler et al., 1998; Hantke & Braun, 2000; Müller et al., 2009; Rabsch et al., 1999; Williams et al., 2006). The iron-siderophore complex is subsequently transported across the inner membrane by an ABC transporter system involving ATP hydrolysis (Crouch et al., 2008; Müller et al., 2009). In the cytoplasm, iron is reduced to ferrous ion (Fe^{2+} or Fe (II)) and released from the siderophore following degradation by the enterobactin esterase Fes (Foster & Spector, 1995; Müller et al., 2009). Furthermore, in *Salmonella* the protein bacterioferritin is responsible for binding and storing excess intracellular iron (Velayudhan et al., 2007).

In the tug-of-war for iron, bacteria must confront an additional host defense mechanism designed to deny access to iron. The antimicrobial peptide known as lipocalin 2, or siderocalin, binds to ferric-enterobactin complexes preventing binding to bacterial OMP receptors (Müller et al., 2009; Nairz et al., 2007; Ratledge, 2007). Siderocalin efficiently stops the growth of many bacteria; however, a few pathogens including serovars of *Salmonella enterica* have found an elegant way around this obstacle. Expression of the pathogen-specific *iroA* gene cluster, composed of the genes *iroBCDEN*, results in a structurally modified form of enterobactin that is no longer recognized by siderocalin (Fischbach, Lin, Zhou, et al., 2006; Müller et al., 2009). Inside the cytoplasm, the *iroB* gene product glucosylates enterobactin forming the new siderophore salmochelin (Bister et al., 2004; Hantke et al., 2003). Salmochelin is then transported across the inner membrane by the *iroC* gene product. After chelating iron, ferric-salmochelin complexes enter the bacterial cell through the OMP channel IroN with energy supplied by the TonB, ExbB, and ExbD tripartite system (Hantke et al., 2003; Müller et al., 2009). Once in the periplasm, ferric-salmochelin is bound by the periplasmic binding protein IroE and delivered to the inner membrane ABC transporter complex (*i.e.*, FepBCDG). Active transport across the inner membrane is followed by IroD-mediated cleavage of salmochelin and release of iron (Müller et al., 2009). Even in the presence of siderocalin, this system allows *Salmonella* access to vital iron. In fact, salmochelins are required for full *Salmonella* virulence in the murine model of infection (Crouch et al., 2008; Raffatellu et al., 2009).

Serovars of *Salmonella enterica* are also able to acquire iron through the uptake of siderophores produced by other organisms. For example, the OMP FhuA allows for uptake of fungal ferrichrome and FoxA mediates transport of ferrioxamine (Killmann et al., 1998; Kingsley et al., 1999). In addition, *S. enterica* serovars appear to be capable of acquiring iron complexed with α-ketoacids and α-hydroxyacids (Kingsley et al., 1996; Reissbrodt et al., 1997). In contrast, salmonellae lack some types of iron transport systems found in other bacteria such as the ferric-citrate transport genes of *E. coli* (Hantke & Braun, 2000; Kingsley et al.,

1999). This suggests that *Salmonella* has attained and/or lost iron acquisition systems as it has evolved and adapted to different host and non-host environments.

2.9.2. Regulation of iron-uptake systems and resistance to Fe(II)-mediated toxicity

In both *Salmonella* and *E. coli*, the repressor protein Fur controls expression of genes involved in siderophore biosynthesis and transport (Hantke & Braun, 1997; Lee & Helmann, 2007; Tsolis et al., 1995). Overall Fur regulates the expression of over 50 genes. Under conditions when iron is plentiful, many of these genes are repressed by Fur. Acting as a repressor, Fur binds to a sequence known as the "Fur box" (Escolar et al., 1998). Under conditions of iron deprivation, Fur repression is lifted and iron uptake systems are induced. Thus, Fur is involved in cytoplasmic Fe(II) homeostasis. Interestingly, the Fur regulon is directly connected to other stress response pathways. For example, as one of the master regulators of the oxidative stress response, OxyR direclty binds to the *fur* promoter inducing its expression (Zheng et al., 1999). The connection between iron stress and oxidative stress is due to the fact that too much iron can be toxic due to an accumulation of hydrogen peroxide and other reactive oxygen species via the Fenton reaction. Not surprisingly, a *fur* null mutant exhibits increased sensitivity to Fe(II)-mediated toxicity (Touati, 2000a). In addition, inactivation of the Fur repressor results in expression of the small regulatory RNA known as RyhB which has global effects on the biogenesis of iron-sulfur clusters, the OxyR regulon, and the σ^S regulon (Benjamin et al., 2010); presumably to counteract the detrimental effects of a *fur* null mutation on cytoplasmic Fe(II) levels.

2.9.3. PmrAB TCS-mediated resistance to Fe(III)-mediated toxicity

As described earlier, the PmrAB TCS is responsive to Fe^{3+} ions (Wosten et al., 2000; Fig. 4). Although long thought to be non-toxic, Chamnongpol et al. (2002) demonstrated that Fe(III) is also toxic but through different mechanisms than Fe(II). Earlier, Wosten et al. (2000) showed that a *pmrA* mutant was hypersensitive to killing by Fe(III) but not to a variety of oxidants. This suggests that PmrAB-regulates resistance mechanisms to Fe(III)-mediated toxicity that are separate from those to Fe(II)-mediated toxicity. This is supported by the findings that a *fur* mutant does not exhibit hypersensitivity to Fe(III) levels (Chamnongpol et al., 2002). What's more, Fe(III) exhibited greater microbicidal activity in a *pmrA* mutant even under anaerobic conditions, indicating that its toxicity occurs independent of oxygen. A possible reason for the hypersensitivity of a *pmrA* null mutant to Fe(III) levels is that Fe(III) appears to increase the permeability of the outer membrane of *pmrA* mutant cells — demonstrated by an increased susceptibility to vancomycin, which normally does not affect Gram-negative bacteria because it is unable to cross the outer membrane. This may correlate with the role of PmrAB in resistance to APs, which also alter membrane permeability. Reconciling PmrAB responsiveness to Fe^{3+} ions and its role in Fe(III) resistance with their roles in AP resistance in hosts – where Fe(III) iron levels are low at best – seems difficult. However, if you consider that PmrAB-regulated functions meet the needs of the bacteria under different conditions then it makes more sense. This is supported by the fact that a *pmrA* mutant is defective in survival within soils (Chamnongpol et al., 2002). This plus the fact that regulation of the PmrAB-regulon *in vivo* likely occurs via the PhoPQ-PmrD pathway suggest that the level of Fe(III) is the environmental cue recognized in non-host environments (*e.g.*, soil) and APs (possibly others) is the signal inducing the PmrAB-regulon in host environments. However, it is clear that one or more of the PmrAB-regulon members are required for resistance to both Fe(III)-mediated and AP-mediated injury to the cell.

2.10. Responses to plant-derived antimicrobial compounds in essential oils

Many organic compounds produced by plants exhibit antimicrobial activity, and there is currently a great deal of interest in using such compounds in foods to inhibit the growth of bacterial pathogens and also to prevent microbial spoilage (Burt, 2004). For example, several compounds from herbal essential oils (EOs) have proven to be effective antimicrobial agents (Burt, 2004; Ceylan & Fung, 2004; Helander et al., 1998). Phenols, terpenes, and aldehydes are among the most active compounds (Ceylan & Fung, 2004). Phenolic compounds appear to play a role in inhibiting enzyme activity, and phenolics and other compounds from EOs are known to disrupt the integrity of cell membranes (Burt, 2004; Ceylan & Fung, 2004). Upon exposure to EO compounds, damage to the bacterial cytoplasmic membrane ultimately results in lethal consequences including leakage of cytosolic constituents and dissipation of pH and electrochemical gradients (Di Pasqua et al., 2007).

Recent evidence has indicated that if exposed to sublethal concentrations of thymol and related compounds, serovars of *Salmonella enterica*, as well as certain other Gram-negative and Gram-positive bacteria, develop resistance, suggesting some bacteria can elicit a stress response to these compounds (Di Pasqua et al., 2010; Dubois-Brissonnet et al., 2011). One component of this stress response appears to involve changes in membrane fatty acid composition upon exposure to sublethal concentrations of terpenes, like thymol, and other organic compounds from EOs (Di Pasqua et al., 2006; Di Pasqua et al., 2007; Dubois-Brissonnet et al., 2011). Alterations in fatty acid saturation, isomerization, and other structural features are induced in response to the membrane stress exerted by these lipid-like compounds (Di Pasqua et al., 2006; Di Pasqua et al., 2007; Dubois-Brissonnet et al., 2011). This stress response appears to be directed against the particular plant-derived compound involved and also differs among bacterial species; although, the overall effect is typically a reduction in unsaturated fatty acids and a corresponding increase in membrane rigidity (Di Pasqua et al., 2006; Di Pasqua et al., 2007).

In addition, proteomic analyses revealed that several proteins, including the heat-shock proteins GroEL and DnaK and the outer-membrane proteins OmpA and OmpX are induced upon low level exposure to thymol; while the expression of several other proteins decreases relative to a control culture (Di Pasqua et al., 2010). These changes are believed to prevent or lessen the membrane damage caused by EO antimicrobial compounds. Furthermore, *S.* Typhimurium adapted to growing in the presence of sublethal concentrations of different types of terpenes develops cross-resistance against other antimicrobial chemicals (Dubois-Brissonnet et al., 2011), indicating that this may represent a more general response of *S.* Typhimurium to the multitude of antimicrobial compounds it may encounter on vegetation and in other natural environments.

3. Conclusions

Serovars of *S. enterica* encounter a variety of stresses while passing between numerous natural, commercial and host environments. These bacteria are incredibly adept and versatile in the strategies they employ to survive within these frequently deleterious environments. *Salmonella* and other bacteria have evolved stress-specific resistance mechanisms that are generated in response to sub-lethal levels of the stress, which increase survival when exposed to higher potentially lethal levels of the stress (*i.e.*, adaptations). Perhaps more remarkably, these bacteria have evolved mechanisms induced by one stress that allow them to resist the hazards of several different, seemingly unrelated, stresses (*i.e.*, general or cross-resistances). These general stress responses might enable the bacteria to anticipate, and prepare for, potentially unfavorable environments they may encounter later, increasing the likelihood of their survival. Thus, salmonellae have evolved multiple complex often interconnected systems of stress management as part of their survival strategies. Control of these overlapping stress response networks are managed by an assortment of regulatory proteins/systems. Integrating these stress responses into a multi-defense strategy allows the organism to anticipate, survive and persist in the various non-host and host environments it encounters. Although much has been learned about how salmonellae, and other enterobacteria, sense and respond to

environmental stresses, there remains a great deal to discover; making microbial stress responses a fruitful and important area of future research.

References

Abshire, K. Z., & Neidhardt, F. C. (1993). Analysis of proteins synthesized by *Salmonella typhimurium* during growth within a host macrophage. *Journal of Bacteriology*, *175*, 3734–3743.

Abouzeed, Y. M., Baucheron, S., & Cloeckaert, A. (2008). *ramR* mutations involved in efflux-mediated multidrug resistance in *Salmonella enterica* serovar Typhimurium. *Antimicrobial Agents and Chemotherapy*, *52*, 2428–2434.

Ades, S. E. (2008). Regulation by destruction: Design of the σ^E envelope stress response. *Current Opinions in Microbiology*, *11*, 535–540.

Adkins, J. N., Mottaz, H. M., Norbeck, A. D., Gustin, J. K., Rue, J., Clauss, T. R., et al. (2006). Analysis of the *Salmonella typhimurium* proteome through environmental response toward infectious conditions. *Molecular & Cellular Proteomics*, *5*, 1450–1461.

Alba, B. M., & Gross, C. A. (2004). Regulation of the *Escherichia coli* sigma-dependent stress response. *Molecular Microbiology*, *52*, 613–619.

Alekshun, M. N., & Levy, S. B. (1997). Regulation of chromosomally mediated multiple antibiotic resistance: The *mar* regulon. *Antimicrobial Agents and Chemotherapy*, *41*, 2067–2075.

Almirón, M., Link, A., Furlong, D., & Kolter, R. (1992). A novel DNA binding protein with regulatory and protective roles in starved *E. coli*. *Genes & Development*, *6*, 2646–2654.

Alpuche-Aranda, C. M., Swanson, J. A., Loomis, W. P., & Miller, S. I. (1992). *Salmonella typhimurium* activates virulence gene transcription within acidified macrophage phagosomes. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 10079–10083.

Altier, C. (2005). Genetic and environmental control of *Salmonella* invasion. *The Journal of Microbiology*, *43*, 85–92.

Altuvia, S., Weinstein-Fischer, D., Zhang, A., Postow, L., & Storz, G. (1997). A small, stable RNA induced by oxidative stress: Role as a pleiotropic regulator and antimutator. *Cell*, *90*, 43–53.

Álvarez-Ordóñez, A., Fernández, A., Bernardo, A., & López, M. (2009). A comparative study of thermal and acid inactivation kinetics in fruit juices of *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Senftenberg grown at acidic conditions. *Foodborne Pathogens and Disease*, *6*, 1147–1155.

Álvarez-Ordóñez, A., Fernández, A., Bernardo, A., & López, M. (2010a). Acid tolerance in *Salmonella typhimurium* induced by culturing in the presence of organic acids at different growth temperatures. *Food Microbiology*, *27*, 44–49.

Álvarez-Ordóñez, A., Fernández, A., Bernardo, A., & López, M. (2010b). Arginine and lysine decarboxylases and the acid tolerance response of *Salmonella* Typhimurium. *International Journal of Food Microbiology*, *136*, 278–282.

Atlung, T., & Ingmer, H. (1997). H-NS: A modulator of environmentally regulated gene expression. *Molecular Microbiology*, *24*, 7–17.

Audia, J. P., & Foster, J. W. (2003). Acid shock accumulation of Sigma S in *Salmonella enterica* involves increased translation, not regulated degradation. *Journal of Molecular Microbiology and Biotechnology*, *5*, 17–28.

Audia, J. P., Webb, C. C., & Foster, J. W. (2001). Breaking through the acid barrier: An orchestrated response to proton stress by enteric bacteria. *International Journal of Medical Microbiology*, *291*, 97–106.

Bader, M. W., Sanowar, S., Daley, M. E., Schneider, A. R., Cho, U., Xu, W., et al. (2005). Recognition of antimicrobial peptides by a bacterial sensor kinase. *Journal of Molecular Biology*, *356*, 1193–1206.

Baik, H. Y., Bearson, S., Dunbar, S., & Foster, J. W. (1996). The acid tolerance response of *Salmonella typhimurium* provides protection against organic acids. *Microbiology*, *142*, 3195–3200.

Bailey, A. M., Ivens, A., Kingsley, R., Cottell, J. L., Wain, J., & Piddock, L. J. (2010). RamA, a member of the AraC/XylS family, influences both virulence and efflux in *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, *192*, 1607–1616.

Balaji, B., O'Conner, K., Lucas, J. R., Anderson, J. M., & Csonka, L. N. (2005). Timing of induction of osmotically controlled genes in *Salmonella enterica* serovar Typhimurium, determined with quantitative real-time reverse transcription-PCR. *Applied and Environmental Microbiology*, *71*, 8273–8283.

Bang, I. S., Kim, B. H., Foster, J. W., & Park, Y. K. (2000). OmpR regulates the stationary-phase acid tolerance response of *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, *182*, 2245–2252.

Bang, I. S., Audia, J. P., Park, Y. K., & Foster, J. W. (2002). Autoinduction of the *ompR* response regulator by acid shock and control of the *Salmonella enterica* acid tolerance response. *Molecular Microbiology*, *44*, 1235–1250.

Bang, I. S., Frye, J. G., McClelland, M., Velayudhan, J., & Fang, F. C. (2005). Alternative sigma factor interactions in *Salmonella*: σ^E and σ^H promote antioxidant defences by enhancing σ^S levels. *Molecular Microbiology*, *56*, 811–823.

Barker, J., & Brown, M. R. W. (1994). Trojan horses of the microbial world: Protozoa and survival of bacterial pathogens in the environment. *Microbiology*, *140*, 1253–1259.

Barnhart, M. M., & Chapman, M. R. (2006). Curli biogenesis and function. *Annual Review of Microbiology*, *60*, 131–147.

Battistoni, A. (2003). Role of prokaryotic Cu, Zn superoxide dismutase in pathogenesis. *Biochemical Society Transactions*, *31*, 1326–1329.

Bäumler, A. J., Norris, T. L., Lasco, T., Voigt, W., Reissbrodt, R., Rabsch, W., et al. (1998). IroN, a novel outer membrane siderophore receptor characteristic of *Salmonella enterica*. *Journal of Bacteriology*, *180*, 1446–1453.

Bearson, S. M., Benjamin, W. H., Swords, W. E., & Foster, J. W. (1996). Acid shock induction of *rpoS* is mediated by the mouse virulence gene *mviA* of *Salmonella typhimurium*. *Journal of Bacteriology*, *178*, 2572–2579.

Bearson, B. L., Wilson, L., & Foster, J. W. (1998). A low pH-inducible PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress. *Journal of Bacteriology*, *180*, 2409–2417.

Bébien, M., Kirsch, J., Méjean, V., & Verméglio, A. (2002). Involvement of a putative molybdenum enzyme in the reduction of selenate by *Escherichia coli*. *Microbiology*, *148*, 3865–3872.

Becker, L. A., Bang, I. S., Crouch, M. L., & Fang, F. C. (2005). Compensatory role of PspA, a member of the phage shock protein operon, in *rpoE* mutant *Salmonella enterica* serovar Typhimurium. *Molecular Microbiology*, *56*, 1004–1016.

Beddek, A. J., & Schryvers, A. B. (2010). The lactoferrin receptor complex in gram negative bacteria. *Biometals*, *23*, 377–386.

Benjamin, J. M., Desnoyers, G., Morissette, A., Salvail, H., & Massé, E. (2010). Dealing with oxidative stress and iron starvation in microorganisms: An overview. *Canadian Journal of Physiology and Pharmacology*, *88*, 264–272.

Berk, P. A., de Jonge, R., Zwietering, M. H., Abee, T., & Kieboom, J. (2005). Acid resistance variability among isolates of *Salmonella enterica* serovar Typhimurium DT 104. *Journal of Applied Microbiology*, *99*, 859–866.

Bhagwat, A. A., Jun, W., Liu, L., Kannan, P., Dharne, M., Pheh, B., et al. (2009). Osmoregulated periplasmic glucans of *Salmonella enterica* serovar Typhimurium are required for optimal virulence in mice. *Microbiology*, *155*, 229–237.

Billi, D., & Potts, M. (2002). Life and death of dried prokaryotes. *Research in Microbiology*, *153*, 7–12.

Bister, B., Bischoff, D., Nicholson, G. J., Valdebenito, M., Schneider, K., Winkelmann, G., et al. (2004). The structure of salmochelins: C-glucosylated enterobactins of *Salmonella enterica*. *Biometals*, *17*, 471–481.

Bohin, J. P. (2000). Osmoregulated periplasmic glucans in proteobacteria. *FEMS Microbiology Letters*, *186*, 11–19.

Boman, H. G., Agerberth, B., & Boman, A. (1993). Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. *Infection and Immunity*, *61*, 2978–2984.

Booth, I. R., & Louis, P. (1999). Managing hypoosmotic stress: aquaporins and mechanosensitive channels in *Escherichia coli*. *Current Opinions in Microbiology*, *2*, 166–169.

Botsford, J. L., Alvarez, M., Hernandez, R., & Nichols, R. (1994). Accumulation of glutamate by *Salmonella typhimurium* in response to osmotic stress. *Applied and Environmental Microbiology*, *60*, 2568–2574.

Bremer, E., & Krämer, R. (2000). Coping with osmotic challenges: Osmoregulation through accumulation and release of compatible solutes in bacteria. In G. Storz, & R. Hengge-Aronis (Eds.), *Bacterial stress responses* (pp. 79–97). Washington, D.C.: ASM Press.

Buchmeier, N. A., Lipps, C. J., So, M. Y. H., & Heffron, F. (1993). Recombination-deficient mutants of *Salmonella typhimurium* are avirulent and sensitive to the oxidative burst of macrophages. *Molecular Microbiology*, *7*, 933–936.

Buchmeier, N. A., Libby, S. J., Xu, Y., Loewen, P. C., Switala, J., Guiney, D. G., et al. (1995). DNA repair is more important than catalase for *Salmonella* virulence in mice. *Journal of Clinical Investigation*, *95*, 1047–1053.

Buchmeier, N. A., Bossie, S., Chen, C. Y., Fang, F. C., Guiney, D. G., & Libby, S. J. (1997). SlyA, a trancriptional regulator of *Salmonella typhimurium*, is required for resistance to oxidative stress and is expressed in the intracellular environment of macrophages. *Infection and Immunity*, *65*, 3725–3730.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods: A review. *International Journal of Food Microbiology*, *94*, 223–253.

Button, J. E., Silhavy, T. J., & Ruiz, N. (2006). A suppressor of cell death caused by the loss of σ^E downregulates extracytoplasmic stress responses and outer membrane vesicle production in *Escherichia coli*. *Journal of Bacteriology*, *189*, 1523–1530.

Cabello, F., Hormaeche, C., Mastroeni, P., & Bonina, L. (Eds.). (1993). *Biology of salmonella*. New York: Plenum Publishing.

Cabiscol, E., Tamarit, J., & Ros, J. (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. *International Microbiology*, *3*, 3–8.

Cadenas, E. (1989). Biochemistry of oxygen toxicity. *Annual Review of Biochemistry*, *58*, 79–110.

Calamita, G., Bishai, W. R., Preston, G. M., Guggino, W. B., & Agre, P. (1995). Molecular cloning and characterization of AqpZ, a water channel from *Escherichia coli*. *Journal of Biological Chemistry*, *270*, 29063–29066.

Campbell, J. W., & Cronan, J. E., Jr. (2002). The enigmatic *Escherichia coli fadE* gene is *yafH*. *Journal of Bacteriology*, *184*, 3759–3764.

Cánovas, D., Fletcher, S. A., Hayashi, M., & Csonka, L. N. (2001). Role of trehalose in growth at high temperature of *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, *183*, 3365–3371.

Carty, S. M., Sreekumar, K. R., & Raetz, C. R. (1999). Effect of cold shock on lipid A biosynthesis in *Escherichia coli*: Induction at 12 °C of an acyltransferase specific for palmitoleoyl-acyl carrier protein. *Journal of Biological Chemistry*, *274*, 9677–9685.

Ceylan, E., & Fung, D. Y. C. (2004). Antimicrobial activity of spices. *Journal of Rapid Methods and Automation in Microbiology*, *12*, 1–55.

Chamnongpol, S., Dodson, W., Cromie, M. J., Harris, Z. L., & Groisman, E. A. (2002). Fe(III)-mediated cellular toxicity. *Molecular Microbiology*, *45*, 711–719.

Cherrington, C. A., Hinton, M., Mead, G. C., & Chopra, I. (1991). Organic acids: Chemistry, antibacterial activity and practical applications. *Advances in Microbial Physiology*, *32*, 87–108.

Christman, M. F., Morgan, R. W., Jacobson, F. S., & Ames, B. N. (1985). Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. *Cell*, *41*, 753–762.

Choi, E., Groisman, E. A., & Shin, D. (2009). Activated by different signals, the PhoP/PhoQ two-component system differentially regulates metal uptake. *Journal of Bacteriology*, *191*, 7174–7181.

Choi, S. K., Park, S. Y., Kim, R., Kim, S. B., Lee, C. H., Kim, J. F., et al. (2009). Identification of a polymyxin synthetase gene cluster of *Paenibacillus polymyxa* and heterologous expression of the gene in *Bacillus subtilis*. *Journal of Bacteriology*, *191*, 3350–3358.

Chu, B. C., Garcia-Herrero, A., Johanson, T. H., Krewulak, K. D., Lau, C. K., Peacock, R. S., et al. (2010). Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. *Biometals*, *23*, 601–611.

Clegg, S. J., Jiam, W., & Cole, J. A. (2006). Role of the *Escherichia coli* nitrate transport protein, NarU, in survival during severe nutrient starvation and slow growth. *Microbiology*, *152*, 2091–2100.

Clements, M., Ericksson, S., Tezcan-Merdol, D., Hinton, J. C. D., & Rhen, M. (2001). Virulence gen regulation in *Salmonella enterica*. *Annals of Medicine*, *33*, 178–185.

Conlin, C. A., & Miller, C. G. (2000). *opdA*, A *Salmonella enterica* serovar Typhimurium gene encoding a protease, is part of an operon regulated by heat shock. *Journal of Bacteriology*, *182*, 518–521.

Cope, L. D., Yogev, R., Miller Eberhard, U., & Hansen, E. J. (1995). A gene cluster involved in the utilization of both free heme and heme: Hemopexin by *Haemophilus influenzae* type b. *Journal of Bacteriology*, *177*, 2644–2653.

Craig, M., & Slauch, J. M. (2009). Phagocytic superoxide specifically damages an extracytoplasmic target to inhibit or kill *Salmonella*. *PloS One*, *4*, 4975–4983.

Crawford, R. W., Gibson, D. L., Kay, W. W., & Gunn, J. S. (2008). Identification of bile-induced exopolysaccharide required for *Salmonella* biofilm formation on gallstone surfaces. *Infection and Immunity*, *76*, 5341–5349.

Crouch, M. L., Becker, L. A., Bang, I. S., Tanabe, H., Oullette, A. J., & Fang, F. C. (2005). The alternative sigma factor sigma E is required for resistance of *Salmonella enterica* serovar Typhimurium to anti-microbial peptides. *Molecular Microbiology*, *56*, 789–799.

Crouch, M. L., Castor, M., Karlinsey, J. E., Kalhorn, T., & Fang, F. C. (2008). Biosynthesis and IroC-dependent export of the siderophore salmochelin are essential for virulence of *Salmonella enterica* serovar Typhimurium. *Molecular Microbiology*, *67*, 971–983.

Crowe, J. H., Hoekstra, F. A., & Crowe, L. M. (1992). Anhydrobiosis. *Annual Review of Physiology*, *54*, 579–599.

Crowe, J. H., Crowe, L. M., Oliver, A. E., Tsvetkova, N., Wolkers, W., & Tablin, F. (2001). The trehalose myth revisited: Introduction to a symposium on stabilization of cells in the dry state. *Cryobiology*, *43*, 89–105.

Dartigalongue, C., Missiakas, D., & Raina, S. (2001). Characterization of the *Escherichia coli* σ^E regulon. *Journal of Biological Chemistry*, *276*, 20866–20875.

Darwin, A. J. (2005). The phage-shock-protein response. *Molecular Microbiology*, *57*, 621–628.

D' Aoust, J. -Y., Maurer, J., & Bailey, J. S. (2001). *Salmonella* species. In M. P. Doyle, L. R. Beuchat, & T. J. Montiville (Eds.), *Food microbiology: Fundamentals and frontiers* (pp. 141–178). (2 edn). Washington, D.C.: ASM Press.

Delamarche, C., Thomas, D., Rolland, J. P., Froger, A., Gouranton, J., Svelto, M., et al. (1999). Visualization of AqpZ-mediated water permeability in *Escherichia coli* by cryoelectron microscopy. *Journal of Bacteriology*, *181*, 4193–4197.

De Las Peñas, A., Connolly, L., & Gross, C. A. (1997a). σ^E is an essential sigma factor in *Escherichia coli*. *Journal of Bacteriology*, *179*, 6862–6864.

De Las Peñas, A., Connolly, L., & Gross, C. A. (1997b). The σ^E-mediated response to extracytoplasmic stress in Escherichia coli is transduced by RseA and RseB, two negative regulators of σ^E. *Molecular Microbiology*, *24*, 373–385.

Delgado, M. A., Mouslim, C., & Groisman, E. A. (2006). The PmrA/PmrB and RcsC/YojN/RcsB systems control expression of the *Salmonella* O-antigen chain length determinant. *Molecular Microbiology*, *60*, 39–50.

Demple, B., & Halbrook, J. (1983). Inducible repair of oxidative DNA damage in *Escherichia coli*. *Nature*, *304*, 466–468.

Demple, B., Hidalgo, E., & Ding, H. (1999). Transcriptional regulation via redox-sensitive iron-sulphur centres in an oxidative stress response. *Biochemical Society Symposia*, *64*, 119–128.

Detweiler, C. S., Monack, D. M., Brodsky, I. E., Mathew, H., & Falkow, S. (2003). *virK*, *soma* and *rscC* are important for systemic *Salmonella enterica* serovar Typhimurium infection and cationic peptide resistance. *Molecular Microbiology*, *48*, 385–400.

De Wulf, P., McGuire, A. M., Liu, X., & Lin, E. C. (2002). Genome-wide profiling of promoter recognition by the two-component response regulator CpxR-P in *Escherichia coli*. *Journal of Biological Chemistry*, *277*, 26652–26661.

Di Pasqua, R., Mamone, G., Ferranti, P., Ercolini, D., & Mauriello, G. (2010). Changes in the proteome of *Salmonella enterica* serovar Thompson as stress adaptation to sublethal concentrations of thymol. *Proteomics*, *10*, 1040–1049.

Di Pasqua, R., Betts, G., Hoskins, N., Edwards, M., Ercolini, D., & Mauriello, G. (2007). Membrane toxicity of antimicrobial compounds from essential oils. *Journal of Agricultural and Food Chemistry*, *55*, 4863–4870.

Di Pasqua, R., Hoskins, N., Betts, G., & Mauriello, G. (2006). Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *Journal of Agricultural and Food Chemistry*, *54*, 2745–2749.

Dodd, C. E. R., Richards, P. J., & Aldsworth, T. G. (2007). Suicide through stress: A bacterial response to sub-lethal injury in the food environment. *International Journal of Food Microbiology*, *120*, 46–50.

Dougherty, T. J., & Pucci, M. J. (1994). Penicillin-binding proteins are regulated by *rpoS* during transitions in growth states of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, *38*, 205–210.

Druilhet, R. E., & Sobek, J. M. (1984). Degradation of cell constituents during starvation of *Salmonella enteritidis*. *Microbios*, *39*, 73–82.

Du, H., Wang, M., Luo, Z., Ni, B., Wang, F., Meng, Y., et al. (2011). Coregulation of gene expression by sigma factors RpoE and RpoS in *Salmonella enterica* serovar Typhi during hyperosmotic stress. *Current Microbiology*, *62*, 1483–1489.

Dubois-Brissonnet, F., Naïtali, M., Mafu, A. A., & Briandet, R. (2011). Induction of fatty acid composition modifications and tolerance to biocides in *Salmonella enterica* serovar Typhimurium by plant-derived terpenes. *Applied and Environmental Microbiology*, *77*, 906–910.

Elbein, A. D., Pan, Y. T., Pastuszak, I., & Carroll, D. (2003). New insights on trehalose: A multifunctional molecule. *Glycobiology*, *13*, 17R–27R.

El-Khani, M. A., & Stretton, R. J. (1981). Effect of growth medium on the lipid composition of log and stationary phase cultures of *Salmonella typhimurium*. *Microbios*, *31*, 161–169.

Empadinhas, N., & da Costa, M. S. (2008). Osmoadaptation mechanisms in prokaryotes: Distribution of compatible solutes. *International Microbiology*, *11*, 151–161.

Erickson, K. D., & Detweiler, C. S. (2006). The Rcs phosphorelay system is specific to enteric pathogens/commensals and activates *ydeI*, a gene important for persistent *Salmonella* infection of mice. *Molecular Microbiology*, *62*, 883–894.

Erickson, J. W., & Gross, C. A. (1989). Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: A second alternate σ factor involved in high-temperature gene expression. *Genes & Development*, *9*, 387–398.

Ernst, R. K., Guina, T., & Miller, S. I. (2001). *Salmonella typhimurium* outer membrane remodeling: Role in resistance to host innate immunity. *Microbes and Infection*, *3*, 1327–1334.

Escolar, L., Perez-Martin, J., & De Lorenzo, V. (1998). Binding of the *fur* (ferric uptake regulator) repressor of *Escherichia coli* to arrays of the GATAAT sequence. *Journal of Molecular Biology*, *283*, 537–547.

Faatz, E., Middendorf, A., & Bremer, E. (1988). Cloned structural genes for the osmotically regulated binding-protein-dependent glycine betaine transport system (ProU) of *Escherichia coli*. *Molecular Microbiology*, *2*, 265–279.

Fang, F. C., Krause, M., Roudier, C., Fierer, J., & Guiney, D. G. (1991). Growth regulation of a *Salmonella* plasmid gene essential for virulence. *Journal of Bacteriology*, *173*, 6783–6789.

Fang, F. C., Libby, S. J., Buchmeier, N. A., Lowen, P. C., Switala, J., Harwood, J., et al. (1992). The alternative σ factor KatF (RpoS) regulates *Salmonella* virulence. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 11978–11982.

Fang, F. C., Chen, C. Y., Guiney, D. G., & Xu, Y. (1996). Identification of σ^S-regulated genes in *Salmonella typhimurium*: Complementary regulatory interactions between σ^S and cyclic AMP receptor protein. *Journal of Bacteriology*, *178*, 5112–5120.

Fang, F. C., Vazquez-Torres, A., & Xu, Y. (1997). The transcriptional regulator SoxS is required for resistance of *Salmonella typhimurium* to paraquat but not for virulence in mice. *Infection and Immunity*, *65*, 5371–5375.

Fang, F. C., DeGroote, M. A., Foster, J. W., Baumler, A. J., Oschsner, U., Testerman, T., et al. (1999). Virulent *Salmonella typhimurium* has two periplasmic Cu, Zn-superoxide dismutases. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 7502–7507.

Fang, F. C., & Rimsky, S. (2008). New insights into transcriptional regulation by H-NS. *Current Opinion in Microbiology*, *11*, 113–120.

Farr, S. B., & Kogoma, T. (1991). Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. *Microbiological Reviews*, *55*, 561–585.

Fass, E., & Groisman, E. A. (2009). Control of *Salmonella* pathogenicity island-2 gene expression. *Current Opinions in Microbiology*, *12*, 199–204.

Fink, R. C., Evans, M. R., Porwollik, S., Vazquez-Torres, A., Jones-Carson, J., Troxell, B., et al. (2007). FNR is a global regulator of virulence and anaerobic metabolism in *Salmonella enterica* serovar Typhimurium (ATCC 14028s). *Journal of Bacteriology*, *189*, 2262–2273.

Finlay, B. B., & Falkow, S. (1989). *Salmonella* as an intracellular parasite. *Molecular Microbiology*, *3*, 1833–1841.

Fischbach, M. A., Lin, H., Liu, D. R., & Walsh, C. T. (2006). How pathogenic bacteria evade mammalian sabotage in the battle for iron. *Nature Chemical Biology*, *2*, 132–138.

Fischbach, M. A., Lin, H., Zhou, L., Yu, Y., Abergel, R. J., Liu, D. R., et al. (2006). The pathogen-associated *iroA* gene cluster mediates bacterial evasion of lipocalin 2. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 16502–16507.

Foster, J. W. (1991). *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. *Journal of Bacteriology*, *173*, 6896–6902.

Foster, J. W. (1993). The acid tolerance response of *Salmonella typhimurium* involves transient synthesis of key acid shock proteins. *Journal of Bacteriology*, *175*, 1981–1987.

Foster, J. W. (1999). When protons attack: Microbial strategies of acid adaptation. *Current Opinions in Microbiology*, *2*, 170–174.

Foster, J. W., & Hall, H. K. (1990). Adaptive acidification tolerance response of *Salmonella typhimurium*. *Journal of Bacteriology*, *172*, 771–778.

Foster, J. W., & Hall, H. K. (1991). Inducible pH-homeostasis and the acid tolerance response of *Salmonella typhimurium*. *Journal of Bacteriology*, *173*, 5129–5135.

Foster, J. W., & Hall, H. K. (1992). The effect of *Salmonella typhimurium* ferric-uptake regulator (*fur*) mutation on iron- and pH-regulated protein synthesis. *Journal of Bacteriology*, *174*, 4317–4323.

Foster, J. W., & Moreno, M. (1999). Inducible acid tolerance mechanisms in enteric bacteria. *Novartis Foundation Symposium*, *221*, 55–74.

Foster, J. W., & Spector, M. P. (1986). Phosphate starvation regulon of *Salmonella typhimurium*. *Journal of Bacteriology*, *166*, 666–669.

Foster, J. W., & Spector, M. P. (1995). How *Salmonella* survive against the odds. *Annual Review of Microbiology*, *49*, 145–174.

França, M. B., Panek, A. D., & Eleutherio, E. C. (2007). Oxidative stress and its effects during dehydration. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *146*, 621–631.

Furuki, T., Oku, K., & Sakurai, M. (2009). Thermodynamic, hydration and structural characteristics of alpha, alpha-trehalose. *Frontiers in Bioscience*, *14*, 3523–3535.

Garmiri, P., Coles, K. E., Humphrey, T. J., & Cogan, T. A. (2008). Role of outer membrane lipopolysaccharides in the protection of *Salmonella enterica* serovar Typhimurium from desiccation damage. *FEMS Microbiology Letters*, *281*, 155–159.

Gennis, R. B., & Stewart, V. (1996). Respiration. In F. C. Neidhardt, R. CurtissIII, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, & H. E. Umbarger (Eds.), *Escherichia coli and salmonella: Cellular and molecular biology* (pp. 217–261). Washington, D.C.: ASM Press.

Gerstel, U., & Römling, U. (2003). The *csgD* promoter, a control unit for biofilm formation in *Salmonella typhimurium*. *Research in Microbiology*, *154*, 659–667.

Ghigo, J. M., Ltoffe, S., & Wandersman, C. (1997). A new type of hemophore-dependent heme acquisition system of *Serratia marcescens* reconstituted in *Escherichia coli*. *Journal of Bacteriology*, *179*, 3572–3579.

Görke, B., & Stülke, J. (2008). Carbon catabolite repression in bacteria: Many ways to make the most out of nutrients. *Nature Reviews Microbiology*, *6*, 613–624.

Grant, A. J., Foster, G. L., McKinley, T. J., Brown, S. P., Clare, S., Maskell, D. J., et al. (2009). Bacterial growth rate and host factors as determinants of intracellular bacterial distributions in systemic *Salmonella enterica* infections. *Infection and Immunity*, *77*, 5608–5611.

Greenberg, J. T., & Demple, B. (1989). A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. *Journal of Bacteriology*, *171*, 3933–3939.

Greenberg, J. T., Monach, P., Chou, J. H., Josephy, P. D., & Demple, B. (1990). Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 6181–6185.

Griffith, K. L., Shah, I. M., & Wolf, R. E., Jr. (2004). Proteolytic degradation of *Escherichia coli* transcriptional activators SoxS and MarA as the mechanism for reversing the induction of the superoxide (SoxRS) and multiple antobiotic resistance (Mar) regulons. *Molecular Microbiology*, *51*, 1801–1816.

Gruzdev, N., Pinto, R., & Sela, S. (2011). Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. *Applied and Environmental Microbiology*, *77*, 1667–1673.

Gu, M., & Imlay, J. A. (2011). The SoxRS response of *Escherichia coli* is directly activated by redox-cycling drugs rather than by superoxide. *Molecular Microbiology*, *79*, 1136–1150.

Guina, T., Yi, E. C., Wang, H., Hackett, M., & Miller, S. I. (2000). A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar Typhimurium promotes resistance to alpha-helical antimicrobial peptides. *Journal of Bacteriology*, *182*, 4077–4086.

Guisbert, E., Herman, C., Lu, C. Z., & Gross, C. A. (2004). A chaperone network controls the heat shock response in *E. coli*. *Genes & Development*, *18*, 2812–2821.

Guisbert, E., Yura, T., Rhodius, V. A., & Gross, C. A. (2008). Convergence of molecular, modeling, and systems approaches for an understanding of the *Escherichia coli* heat shock response. *Microbiology and Molecular Biology Reviews*, *72*, 545–554.

Gunn, J. S. (2000). Mechanisms of bacterial resistance and response to bile. *Microbes and Infection*, *2*, 907–913.

Gunn, J. S. (2008). The *Salmonella* PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. *Trends in Microbiology*, *16*, 284–290.

Gunn, J. S., Ryan, S. S., Van Velkinburgh, J. C., Ernst, R. K., & Miller, S. I. (2000). Genetic and functional analysis of a PmrA-PmrB-regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica* serovar Typhimurium. *Infection and Immunity*, *68*, 6139–6146.

Guo, L., Lim, K. B., Poduje, C. M., Daniel, M., Gunn, J. S., Hackett, M., et al. (1998). Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. *Cell*, *95*, 189–198.

Gutsmann, T., Hagge, S. O., Larrick, J. W., Seydel, U., & Wiese, A. (2001). Interaction of CAP18-derived peptides with membranes made from endotoxins or phospholipids. *Biophysical Journal*, *80*, 2935–2945.

Haiko, J., Suomalainen, M., Ojala, T., Lähteenmäki, K., & Korhonen, T. K. (2009). Breaking barriers — attack on innate immune defences by omptin surface proteases of enterobacterial pathogens. *Innate Immunity*, *15*, 67–80.

Hall, H. K., & Foster, J. W. (1996). The role of Fur in the acid tolerance response of *Salmonella typhimurium* is physiologically and genetically separable from its role in iron acquisition. *Journal of Bacteriology*, *178*, 5683–5691.

Hansen, A. M., Qiu, Y., Yeh, N., Blattner, F. R., Durfee, T., & Jin, D. J. (2005). SspA is required for acid resistance in stationary phase by downregulation of H-NS in *Escherichia coli*. *Molecular Microbiology*, *56*, 719–734.

Hantke, K., & Braun, V. (1997). Control of bacterial iron transport by regulatory proteins. In S. Silver, & W. Walden (Eds.), *Metal ions in gene regulation* (pp. 11–44). New York: Chapman and Hall.

Hantke, K., & Braun, V. (2000). The art of keeping low and high iron concentrations in balance. In G. Storz, & R. Hengge-Aronis (Eds.), *Bacterial stress responses* (pp. 275–288). Washington, D.C.: ASM Press.

Hantke, K., Nicholson, G., Rabsch, W., & Winkelmann, G. (2003). Salmochelins, siderophores of *Salmonella enterica* and uropathogenic *Escherichia coli* strains, are recognized by the outer membrane receptor IroN. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 3677–3682.

Harder, W., & Dijkhuizen, L. (1983). Physiological responses to nutrient limitation. *Annual Review of Microbiology*, *37*, 1–23.

Hassett, D. J., & Cohen, M. S. (1989). Bacterial adaptation to oxidative stress: Implications for pathogenesis and interaction with phagocytic cells. *The FASEB Journal*, *3*, 2574–2582.

Hébrard, M., Viala, J. P., Méresse, S., Barras, F., & Aussel, L. (2009). Redundant hydrogen peroxide scavengers contribute to *Salmonella* virulence and oxidative stress resistance. *Journal of Bacteriology*, *191*, 4605–4614.

Heithoff, D. M., Conner, C. P., Hentschel, U., Govantes, F., Hanna, P. C., & Mahan, M. J. (1999). Coordinate intracellular expression of *Salmonella* genes induced during infection. *Journal of Bacteriology*, *181*, 799–807.

Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., et al. (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. *Journal of Agricultural and Food Chemistry*, *46*, 3590–3595.

Helmann, J. D. (2002). OxyR: A molecular code for redox sensing? *Science Signalling Transduction Knowledge Environment*, *157*, 46.

Henard, C. A., Bourret, T. J., Song, M., & Vázquez-Torres, A. (2010). Control of redox balance by the stringent response regulatory protein promotes antioxidant defenses of *Salmonella*. *Journal of Biological Chemistry*, *285*, 36785–36793.

Henderson, T. A., Dombrosky, P. M., & Young, K. D. (1994). Artifactual processing of penicillin-binding protein 7 and 1b by the OmpT protease of *Escherichia coli*. *Journal of Bacteriology*, *176*, 256–259.

Henderson, T. A., Templin, M., & Young, K. D. (1995). Identification and cloning of the gene encoding penicillin-binding protein 7 of *Escherichia coli*. *Journal of Bacteriology*, *177*, 2074–2079.

Hengge, R. (2008). The two-component network and the general stress sigma factor RpoS (σ^S) in *Escherichia coli*. *Advances in Experimental Medicine and Biology*, *631*, 40–53.

Hengge-Aronis, R. (1999). Interplay of global regulators and cell physiology in the general stress response of *Escherichia coli*. *Current Opinions in Microbiology*, *2*, 148–152.

Hengge-Aronis, R. (2002). Signal transduction and regulatory mechansism invovled in control of the σ^S (RpoS) subunit of RNA polymerase. *Microbiology and Molecular Biology Reviews*, *66*, 373–395.

Herman, C., Thevenet, D., D'Ari, R., & Bouloc, P. (1995). Degradation of σ^{32}, the heat shock regulator in *Escherichia coli*, is governed by HflB. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 3516–3520.

Howells, A. M., Bullifent, H. L., Dhaliwal, K., Griffin, K., de Castro, A. G., Frith, G., et al. (2002). Role of trehalose biosynthesis in environmental survival and virulence of *Salmonella enterica* serovar Typhimurium. *Research in Microbiology*, *153*, 281–287.

Huisman, G. W., Seigele, D. A., Zambrano, M. M., & Kolter, R. (1996). Morphological and physiological changes during stationary phase. In F. C. Neidhardt, R. CurtissIII, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, & H. E. Umbarger (Eds.), *Escherichia coli and salmonella: Cellular and molecular biology* (pp. 1672–1682). Washington, D.C.: ASM Press.

Humphreys, S., Stevenson, A., Bacon, A., Weinhardt, A. B., & Roberts, M. (1999). The alternative sigma factor, σ^E, is critically important for the virulence of *Salmonella typhimurium*. *Infection and Immunity*, *67*, 1560–1568.

Humphreys, S., Rowley, G., Stevenson, A., Kenyon, W. J., Spector, M. P., & Roberts, M. (2003). Role of periplasmic peptidylprolyl isomerases in *Salmonella enterica* serovar Typhimurium virulence. *Infection and Immunity*, *71*, 5386–5388.

Humphreys, S., Rowley, G., Stevenson, A., Anjum, M. F., Woodward, M. J., Gilbert, S., et al. (2004). Role of the two-component regulator CpxAR in the virulence of *Salmonella enterica* serotype Typhimurium. *Infection and Immunity*, *72*, 4654–4661.

Ibanez-Ruiz, M., Robbe-Saule, V., Hermant, D., Labrude, S., & Norel, F. (2000). Identification of RpoS (σ^S)-regulated genes in *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, *182*, 5749–5756.

Inbaraj, J. J., & Chignell, C. F. (2004). Cytotoxic action of juglone and plumbagin: A mechanistic study using HaCaT keratinocytes. *Chemical Research in Toxicology*, *17*, 55–62.

Imlay, J. A. (2008). Cellular defenses against superoxide and hydrogen peroxide. *Annual Review of Biochemistry*, *77*, 755–756.

Jain, S., & Chen, J. (2007). Attachment and biofilm formation by various serotypes of *Salmonella* as influenced by cellulose production and thin aggregative fimbriae biosynthesis. *Journal of Food Protection*, *70*, 2473–2479.

Janssen, R., van der Straaten, T., van Diepen, A., & van Dissel, J. T. (2003). Responses to reactive oxygen intermediates and virulence of *Salmonella typhimurium*. *Microbes and Infection*, *5*, 527–534.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern food microbiology* (7th edn.). New York: Springer.

Jones, A. M., Goodwill, A., & Thomas Elliott, T. (2006). Limited role for the DsrA and RprA regulatory RNAs in *rpoS* regulation in *Salmonella enterica*. *Journal of Bacteriology*, *188*, 5077–5088.

Kaasen, I., Falkenberg, P., Styrvold, O. B., & Strøm, A. R. (1992). Molecular cloning and physical mapping of the *otsBA* genes, which encode the osmoregulatory trehalose pathway of *Escherichia coli*: evidence that transcription is activated by KatF (AppR). *Journal of Bacteriology*, *174*, 889–898.

Kanemori, M., Yanagi, H., & Yura, T. (1999). Marked instability of the σ^{32} heat shock transcription factor at high temperature: implications on the heat shock regulation. *Journal of Biological Chemistry*, *274*, 22002–22007.

Karatzas, K. A. G., Hocking, P. M., Jørgensen, F., Mattick, K., Leach, S., & Humphreys, T. J. (2008). Effects of repeated cycles of acid challenge and growth on the phenotype and virulence of *Salmonella enterica*. *Journal of Applied Microbiology*, *105*, 1640–1648.

Kato, A., & Groisman, E. A. (2008). The PhoQ/PhoP regulatory network of *Salmonella enterica*. *Advances in Experimental Medicine and Biology*, *631*, 7–21.

Kenyon, W. J., & Spector, M. P. (2011). Response of *Salmonella enterica* serovars to environmental stresses. In Hin-chung Wong (Ed.), *Stress responses in foodborne microorganisms*. Hauppauge, NY: Nova Science Publishing, Inc.

Kenyon, W. J., Sayers, D. G., Humphreys, S., Roberts, M., & Spector, M. P. (2002). The starvation-stress response of *Salmonella enterica* serovar Typhimurium requires σ^E-, but not CpxR-regulated extracytoplasmic functions. *Microbiology*, *148*, 113–122.

Kenyon, W. J., Thomas, S. M., Johnson, E., Pallen, M., & Spector, M. P. (2005). Shifts from glucose to certain secondary carbon-sources result in activation of the extra-cytoplasmic function sigma factor σ^E in *Salmonella enterica* serovar Typhimurium. *Microbiology*, *151*, 2373–2383.

Kenyon, W. J., Nicholson, K. L., Rezuchova, B., Homerova, D., Garcia del-Portillo, F., Finlay, B. B., et al. (2007). σ^S-Dependent carbon-starvation-induction of *pbpG* (PBP 7) is required for the starvation-stress response in *Salmonella enterica* serovar Typhimurium. *Microbiology*, *153*, 2148–2158.

Kenyon, W. J., Humphreys, S., Roberts, M., & Spector, M. P. (2010). Periplasmic peptidyl-prolyl isomerases SurA and FkpA play an important role in the starvation-stress response (SSR) of *Salmonella enterica* serovar Typhimurium. *Antonie Van Leeuwenhoek*, *98*, 51–63.

Kieboom, J., & Abee, T. (2006). Arginine-dependent acid resistance in *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, *188*, 5650–5653.

Killmann, H., Herrmann, C., Wolff, H., & Braun, V. (1998). Identification of a new site for ferrichrome transport by comparison of the FhuA proteins of *Escherichia coli*, *Salmonella paratyphi* B, *Salmonella typhimurium*, and *Pantoea agglomerans*. *Journal of Bacteriology*, *180*, 3845–3852.

Kim, S. O., Merchant, K., Nudelman, R., Beyer, W. F., Jr., Keng, T., DeAngelo, J., et al. (2002). OxyR: A molecular code for redox-related signaling. *Cell*, *109*, 383–396.

Kingsley, R., Rabsch, W., Roberts, M., Reissbrodt, R., & Williams, P. H. (1996). TonB-dependent iron supply in *Salmonella* by alpha-ketoacids and alpha-hydroxyacids. *FEMS Microbiology Letters*, *140*, 65–70.

Kingsley, R. A., Reissbrodt, R., Rabsch, W., Ketley, J. M., Tsolis, R. M., Everest, P., et al. (1999). Ferrioxamine-mediated Iron(III) utilization by *Salmonella enterica*. *Applied and Environmental Microbiology*, *65*, 1610–1618.

Kleerebezem, M., Crielaard, W., & Tommassen, J. (1996). Involvement of stress protein PspA (phage shock protein A) of *Escherichia coli* in maintenance of the proton motive force under stress conditions. *EMBO Journal*, *15*, 162–171.

Koch, A. L. (1971). The adaptive response of *Escherichia coli* to a feast or famine existence. *Advances in Microbial Physiology*, *6*, 147–217.

Koo, M. S., Lee, J. H., Rah, S. Y., Yeo, W. S., Lee, J. W., Lee, K. L., et al. (2003). A reducing system of the superoxide sensor SoxR in *Escherichia coli*. *EMBO Journal*, *22*, 2614–2622.

Kung, C., Martinac, B., & Sukharev, S. (2010). Mechanosensitive channels in microbes. *Annual Reviews in Microbiology*, *64*, 313–329.

Kwon, Y. M., & Ricke, S. C. (1998). Induction of acid resistance of *Salmonella typhimurium* by exposure to short-chain fatty acids. *Applied and Environmental Microbiology*, *64*, 3458–3463.

Leblanc, S. K., Oates, C. W., & Raivio, T. L. (2011). Characterization of the induction and cellular role of the BaeSR two-component envelope stress response of *Escherichia coli*. *Journal of Bacteriology*, *193*, 3367–3375.

Leclerc, G. J., Tartera, C., & Metcalf, E. S. (1998). Environmental regulation of *Salmonella typhi* invasion-defective mutants. *Infection and Immunity*, *66*, 682–691.

Lee, I. S., Slonczewski, J. L., & Foster, J. W. (1994). A low pH-inducible stationary-phase acid tolerance response in *Salmonella typhimurium*. *Journal of Bacteriology*, *176*, 1422–1426.

Lee, I. S., Lin, J., Hall, H. K., Bearson, B., & Foster, J. W. (1995). The stationary-phase sigma factor σ^S (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium*. *Molecular Microbiology*, *17*, 155–167.

Lee, J. W., & Helmann, J. D. (2007). Functional specialization within the Fur family of metalloregulators. *Biometals*, *20*, 485–499.

Lee, S., Cho, E., & Jung, S. (2009). Periplasmic glucans isolated from proteobacteria. *BMB Reports*, *42*, 769–775.

Lettofe, S., Redeker, V., & Wandersman, C. (1998). Isolation and characterization of an extracellular haem-binding protein from *Pseudomonas aeruginosa* that shares function and sequence similarities with the *Serratia marcescens* HasA haemophore. *Molecular Microbiology*, *28*, 1223–1234.

Levina, N., Tötemeyer, S., Stokes, N. R., Louis, P., Jones, M. A., & Booth, I. R. (1999). Protection of *Escherichia coli* cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: Identification of genes required for MscS activity. *EMBO Journal*, *18*, 1730–1737.

Lewis, C., Skovierova, H., Rowley, G., Rezuchova, B., Homerova, D., Stevenson, A., et al. (2009). *Salmonella enterica* serovar Typhimurium HtrA: Regulation of expression and role of the chaperone and protease activities during infection. *Microbiology*, *155*, 873–881.

Leyer, G., & Johnson, E. A. (1993). Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. *Applied and Environmental Microbiology*, *59*, 1842–1847.

Lin, J., Lee, I. S., Frey, J., Slonczewski, J. L., & Foster, J. W. (1995). Comparative analysis of extreme acid survival in *Salmonella typhimurium*, *Shigella flexneri* and *Escherichia coli*. *Journal of Bacteriology*, *177*, 4097–4104.

Liu, L., Tan, S., Jun, W., Smith, A., Meng, J., & Bhagwat, A. A. (2009). Osmoregulated periplasmic glucans are needed for competitive growth and biofilm formation by *Salmonella enterica* serovar Typhimurium in leafy-green vegetable wash waters and colonization in mice. *FEMS Microbiology Letters*, *292*, 13–20.

López-Garrido, J., Cheng, N., García-Quintanilla, F., García-del Portillo, F., & Casadesús, J. (2010). Identification of the *Salmonella enterica damX* gene product, an inner membrane protein involved in bile resistance. *Journal of Bacteriology*, *192*, 893–895.

Lu, S., Killoran, P. B., Fang, F. C., & Riley, L. W. (2002). The global regulator ArcA controls resistance to reactive nitrogen and oxygen intermediates in *Salmonella enterica* serovar Enteriditis. *Infection and Immunity*, *70*, 451–461.

Lucas, R. L., & Lee, C. A. (2000). Unraveling the mysteries of virulence gene regulation in *Salmonella typhimurium*. *Molecular Microbiology*, *36*, 1024–1033.

Lucchini, S., McDermott, P., Thompson, A., & Hinton, J. C. D. (2009). The H-NS-like protein StpA represses the RpoS (σ^{38}) regulon during exponential growth of *Salmonella* Typhimurium. *Molecular Microbiology*, *74*, 1169–1186.

Lund, P. A. (2001). Microbial molecular chaperones. *Advances in Microbial Physiology*, *44*, 93–140.

MacRitchie, D. M., Buelow, D. R., Price, N. L., & Raivio, T. L. (2008). Two-component signaling and Gram negative envelope stress response systems. In R. Utsumi (Ed.), *Advances in Experimental Medicine and Biology: BacterialSignal Transduction: Networks and Drug Targets*, *631*. (pp. 80–110).

Matin, A. (2009). Stress, bacterial: General and specific. In M. Schaechter (Ed.), *The desk encyclopedia of microbiology* (pp. 1075–1090). (2nd edn.). Oxford, UK: Elsevier, Inc.

McFall, E., & Newman, E. B. (1996). Amino acids as carbon sources. In F. C. Neidhardt, R. CurtissIII, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, & H. E. Umbarger (Eds.), *Escherichia coli and salmonella: Cellular and molecular biology* (pp. 358–379). Washington, D.C.: ASM Press.

McLeod, G. I., & Spector, M. P. (1996). Starvation and stationary-phase induced resistance to the antimicrobial peptide polymyxin B is RpoS-independent and occurs through both *phoP*-dependent and *phoP*-independent pathways in *Salmonella typhimurium*. *Journal of Bacteriology*, *178*, 3683–3688.

McMahon, M. A. S., Xu, J., Moore, J. E., Blair, I. S., & McDowell, D. A. (2007). Environmental stress and antibiotic resistance in food-related pathogens. *Applied and Environmental Microbiology*, *73*, 211–217.

McMahon, M. A. S., McDowell, D. A., & Blair, I. S. (2007). The pattern of pleiomorphism in stressed *Salmonella* Virchow populations is nutrient and growth phase dependent. *Letters in Applied Microbiology*, *45*, 276–281.

McMeechan, A., Roberts, M., Cogan, T. A., Jørgensen, F., Stevenson, A., Lewis, C., et al. (2007). Role of the alternative sigma factors σ^E and σ^S in survival of *Salmonella enterica* serovar Typhimurium during starvation, refrigeration and osmotic shock. *Microbiology*, *153*, 236–269.

Meberg, B. M., Paulson, A. L., Priyadarshini, R., & Young, K. D. (2004). Endopeptidase penicillin-binding proteins 4 and 7 play auxiliary roles in determining uniform morphology of *Escherichia coli*. *Journal of Bacteriology*, *186*, 8326–8336.

Merighi, M., Ellermeier, C. D., Slauch, J. M., & Gunn, J. S. (2005). Resolvase-in vivo expression technology analysis of the *Salmonella enterica* serovar Typhimurium PhoP and PmrA regulons in BALB/c mice. *Journal of Bacteriology*, *187*, 7407–7416.

Merritt, M. E., & Donaldson, J. R. (2009). Effect of bile salts on the DNA and membrane intergrity of enteric bacteria. *Journal of Medical Microbiology*, *58*, 1533–1541.

Michán, C., Manchado, M., Dorado, G., & Pueyo, C. (1999). In vivo transcription of the *Escherichia coli oxyR* regulon as a function of growth phase and in response to oxidative stress. *Journal of Bacteriology*, *181*, 2759–2764.

Misra, R., & Miao, Y. (1995). Molecular analysis of *asmA*, a locus identified as a suppressor of OmpF assembly mutants in *Escherichia coli* K-12. *Molecular Microbiology*, *16*, 779–788.

Missiakas, D., Mayer, M. P., Lemaire, M., Georgopoulos, C., & Raina, S. (1997). Modulation of the *Escherichia coli* σ^E (RpoE) heat-shock transcription-factor activity by the RseA, RseB and RseC proteins. *Molecular Microbiology*, *24*, 355–371.

Mizuno, T., & Mizushima, S. (1990). Signal transduction and gene regulation through the phosphorylation of two regulatory components: The molecular basis for the osmotic regulation of the porin genes. *Molecular Microbiology*, *4*, 1077–1082.

Morbach, S., & Krämer, R. (2002). Body shaping under water stress: Osmosensing and osmoregulation of solute transport in bacteria. *ChemBioChem*, *3*, 384–397.

Morgan, R. W., Christman, M. F., Jacobson, F. S., Storz, G., & Ames, B. N. (1986). Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *83*, 8059–8063.

Morita, M., Kanemori, M., Yanagi, H., & Yura, T. (1999). Heat-induced synthesis of σ^{32} in *Escherichia coli*: Structural and functional dissection of *rpoH* mRNA secondary structure. *Journal of Bacteriology*, *181*, 401–410.

Morita, M. T., Tanaka, Y., Kodama, T. S., Kyogoku, Y., Yanagi, H., & Yura, T. (1999). Translational induction of heat shock transcription factor σ^{32}: Evidence for a built-in RNA thermosensor. *Genes & Development*, *13*, 655–665.

Morita, M. T., Kanemori, M., Yanagi, H., & Yura, T. (2000). Dynamic interplay between antagonistic pathways controlling the σ^{32} level in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 5860–5865.

Müller, S. I., Valdebenito, M., & Hantke, K. (2009). Salmochelin, the long-overlooked catecholate siderophore of *Salmonella*. *Biometals*, *22*, 691–695.

Nakayama, S., Kushiro, A., Asahara, T., Tanaka, R., Hu, L., Kopecko, D. J., et al. (2003). Activation of *hilA* expression at low pH requires the signal sensor CpxA, but not the cognate response regulator CpxR, in *Salmonella enterica* serovar Typhimurium. *Microbiology*, *149*, 2809–2817.

Nairz, M., Theurl, I., Ludwiczek, S., Theurl, M., Mair, S. M., Fritsche, G., et al. (2007). The co-ordinated regulation of iron homeostasis in murine macrophages limits the availability of iron for intracellular *Salmonella typhimurium*. *Cellular Microbiology*, *9*, 2126–2140.

Navarre, W. W., Halsey, T. A., Walthers, D., Frye, J., McClelland, M., Potter, J. L., et al. (2005). Co-regulation of *Salmonella enterica* genes required for virulence and resistance to antimicrobial peptides by SlyA and PhoP/PhoQ. *Molecular Microbiology*, *56*, 492–508.

Navarro Llorens, J. M., Tormo, A., & Martínez-García, E. (2010). Stationary phase in gram-negative bacteria. *FEMS Microbiology Reviews*, *34*, 476–495.

Nikaido, E., Yamaguchi, A., & Nishino, K. E. (2008). AcrAB multidrug efflux pump regulation in *Salmonella enterica* serovar Typhimurium by RamA in response to environmental signals. *Journal of Biological Chemistry*, *283*, 24245–24253.

Nishino, K., Honda, T., & Yamaguchi, A. (2005). Genome-wide analyses of *Escherichia coli* gene expression responsive to the BaeSR two-component regulatory system. *Journal of Bacteriology*, *187*, 1763–1772.

Nishino, K., Latifi, T., & Groisman, E. A. (2006). Virulence and drug resistance roles of multidrug efflux systems of *Salmonella enterica* serovar Typhimurium. *Molecular Microbiology*, *59*, 126–141.

Nishino, K., Nikaido, E., & Yamaguchi, A. (2007). Regulation of multidrug efflux systems involved in multidrug and metal resistance of *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, *189*, 9066–9075.

Nishino, K., Nikaido, E., & Yamaguchi, A. (2009). Regulation and physiological function of multidrug efflux pumps in *Escherichia coli* and *Salmonella*. *Biochimica et Biophysica Acta — Proteins and Proteomics*, *1794*, 834–843.

Nizet, V. (2006). Antimicrobial peptide resistance mechanisms of human bacterial pathogens. *Current Issues in Molecular Biology*, *8*, 11–26.

Nonaka, G., Blankschien, M., Herman, C., Gross, C. A., & Rhodius, V. A. (2006). Regulon and promoter analysis of the *E. coli* heat-shock factor, σ^{32}, reveals a multifaceted cellular response to heat stress. *Genes & Development*, *20*, 1776–1789.

Nunoshiba, T., Hidalgo, E., Amábile-Cuevas, C. F., & Demple, B. (1992). Two-stage control of an oxidative stress regulon: The *Escherichia coli* SoxR protein triggers redox-inducible expression of the *soxS* regulatory gene. *Journal of Bacteriology*, *174*, 6054–6060.

Nunoshiba, T., DeRojas-Walker, T., Wishnok, J. S., Tannenbaum, S. R., & Demple, B. (1993). Activation by nitric oxide of an oxidative-stress response that defends *Escherichia coli* against activated macrophages. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 9993–9997.

Nunoshiba, T., DeRojas-Walker, T., Tannenbaum, S. R., & Demple, B. (1995). Roles of nitric oxide in inducible resistance of *Escherichia coli* to activated murine macrophages. *Infection and Immunity*, *63*, 794–798.

Nyström, T., Larsson, C., & Gustafsson, L. (1996). Bacterial defense against aging: role of the *Escherichia coli* ArcA regulator in gene expression, readjusted energy flux and survival during stasis. *EMBO Journal*, *15*, 3219–3228.

Oh, Y. K., Alpuche-Aranda, C., Berthiaume, E., Jinks, T., Miller, S. I., & Swanson, J. A. (1996). Rapid and complete fusion of macrophage lysosomes with phagosomes containing *Salmonella typhimurium*. *Infection and Immunity*, *64*, 3877–3883.

O'Neal, C. R., Gabriel, W. M., Turk, A. K., Fang, F. C., Libby, S., & Spector, M. P. (1994). RpoS is necessary for both the positive and negative regulation of starvation-survival genes during phosphate-, carbon-, and nitrogen-starvation in *Salmonella typhimurium*. *Journal of Bacteriology*, *176*, 4610–4616.

Ono, S., Goldberg, M. D., Olsson, T., Esposito, D., Hinton, J. C., & Ladbury, J. E. (2005). H-NS is part of a thermally controlled mechanism for bacterial gene regulation. *Biochemical Journal*, *391*, 203–213.

Ophir, T., & Gutnick, D. L. (1994). A role for exopolysaccharides in the protection of microorganisms from desiccation. *Applied and Environmental Microbiology*, *60*, 740–745.

Pálffy, R., Gardlík, R., Behuliak, M., Kadasi, L., Turna, J., & Celec, P. (2009). On the physiology and pathophysiology of antimicrobial peptides. *Molecular Medicine*, *15*, 51–59.

Papp-Szabò, E., Firtel, M., & Josephy, P. D. (1994). Comparison of the sensitivities of *Salmonella typhimurium oxyR* and *katG* mutants to killing by human neutrophils. *Infection and Immunity*, *62*, 2662–2668.

Park, K. R., Giard, J. C., Eom, J. H., Bearson, S., & Foster, J. W. (1998). Cyclic AMP receptor protein and TyrR are required for acid pH and anaerobic induction of *hyaB* and *aniC* in *Salmonella typhimurium*. *Journal of Bacteriology*, *181*, 689–694.

Park, Y. K., Bearson, B., Bang, S. H., Bang, I. S., & Foster, J. W. (1996). Internal pH crisis, lysine decarboxylase and the acid tolerance response of *Salmonella typhimurium*. *Molecular Microbiology*, *20*, 605–611.

Paterson, G. K., Northen, H., Cone, D. B., Willers, C., Peters, S. E., & Maskell, D. J. (2009). Deletion of *tolA* in *Salmonella* Typhimurium generates an attenuated strain with vaccine potential. *Microbiology*, *155*, 220–228.

Perez, J. C., Shin, D., Zwir, I., Latifi, T., Hadley, T. J., & Groisman, E. A. (2009). Evolution of a bacterial regulon controlling virulence and Mg^{2+} homeostasis. *PLoS Genetics*, *5*, e1000428.

Peschel, A. (2002). How do bacteria resist human antimicrobial peptides? *Trends in Microbiology*, *10*, 179–186.

Pilonieta, M. C., Erickson, K. D., Ernst, R. K., & Detweiler, C. S. (2009). A protein important for antimicrobial peptide resistance, YdeI/OmdA, is in the periplasm and interacts with OmpD/NmpC. *Journal of Bacteriology*, *191*, 7243–7252.

Pollack, J. R., & Neilands, J. B. (1970). Enterobactin, an iron transport compound from *Salmonella typhimurium*. *Biochemical and Biophysical Research Communications*, *38*, 982–989.

Pomposiello, P. J., & Demple, B. (2000). Identification of SoxS-regulated genes in *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, *182*, 23–29.

Pomposiello, P. J., & Demple, B. (2001). Redox-operated genetic switches: The SoxR and OxyR transcription factors. *Trends in Biotechnology*, *19*, 109–114.

Pompsiello, P. J., Bennik, M. H., & Demple, B. (2001). Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. *Journal of Bacteriology*, *183*, 3890–3902.

Poolman, B., Blount, P., Folgering, J. H. A., Friesen, R. H. E., Moe, P. C., & van der Heide, T. (2002). How do membrane proteins sense water stress? *Molecular Microbiology*, *44*, 889–902.

Potrykus, K., & Cashel, M. (2008). (p)ppGpp still magical? *Annual Review of Microbiology*, *62*, 35–51.

Potts, M. (1994). Desiccation tolerance of prokaryotes. *Microbiological Reviews*, *58*, 755–805.

Potts, M. (2001). Desiccation tolerance: A simple process? *Trends in Microbiology*, *9*, 553–559.

Pratt, L. A., & Silhavy, T. J. (1996). From acids to *osmZ*: Multiple factors influence synthesis of the OmpF and OmpC porins in *Escherichia coli*. *Molecular Microbiology*, *20*, 911–917.

Price, N. L., & Raivio, T. L. (2009). Characterization of the Cpx regulon in *Escherichia coli* strain MC4100. *Journal of Bacteriology*, *191*, 1798–1815.

Prieto, A. I., Ramos-Morales, F., & Casadesús, J. (2006). Repair of DNA damage induced by bile salts in *Salmonella enterica*. *Genetics*, *174*, 575–584.

Prieto, A. I., Hernández, S. B., Costa, I., Pucciarelli, M. G., Orlov, Y., Ramos-Morales, F., et al. (2009). Roles of the outer membrane protein AsmA of *Salmonella enterica* in the control of *marRAB* expression and invasion of epithelial cells. *Journal of Bacteriology*, *191*, 3615–3622.

Prost, L. R., & Miller, S. I. (2008). The Salmonellae PhoQ sensor: Mechanisms of detection of phagosome signals. *Cellular Microbiology*, *10*, 576–582.

Prost, L. R., Sanowar, S., & Miller, S. I. (2007). *Salmonella* sensing of anti-microbial mechanisms to promote survival within macrophages. *Immunological Reviews*, *219*, 55–65.

Prouty, A. M., Van Velkinburgh, J. C., & Gunn, J. S. (2002). *Salmonella enterica* serovar Typhimurium resistance to bile: Identification and characterization of the *tolQRA* cluster. *Journal of Bacteriology*, *184*, 1270–1276.

Prouty, A. M., Schwesinger, W. H., & Gunn, J. S. (2002). Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp. *Infection and Immunity*, *70*, 2640–2649.

Prouty, A. M., Brodsky, I. E., Falkow, S., & Gunn, J. S. (2004). Bile salt-mediated induction of antimicrobial and bile resistance in *Salmonella typhimurium*. *Microbiology*, *150*, 775–783.

Prouty, A. M., Brodsky, I. E., Manos, J., Belas, F., Falkow, S., & Gunn, J. S. (2004). Transcriptional regulation of *Salmonella enterica* serovar Typhimurium genes by bile. *FEMS Immunology and Medical Microbiology*, *41*, 177–185.

Rabsch, W., Voigt, W., Reissbrodt, R., Tsolis, R. M., & Braumler, A. J. (1999). *Salmonella typhimurium* IroN and FepA proteins mediate uptake of enterobactin but differ in their specificity for other siderophores. *Journal of Bacteriology*, *181*, 3610–3612.

Raffa, R. G., & Raivio, T. L. (2002). A third envelope stress signal tranduction pathway in *Escherichia coli*. *Molecular Microbiology*, *45*, 1599–1611.

Raffatellu, M., George, M. D., Akiyama, Y., Hornsby, M. J., Nuccio, S. P., Paixao, T. A., et al. (2009). Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype Typhimurium for growth and survival in the inflamed intestine. *Cell Host & Microbe*, *5*, 476–486.

Rathman, M., Sjaastad, M. D., & Falkow, S. (1996). Acidification of phagosomes containing *Salmonella typhimurium* is murine macrophages. *Infection and Immunity*, *64*, 2765–2773.

Ratledge, C. (2007). Iron metabolism and infection. *Food and Nutrition Bulletin*, *28*, S515–S523.

Raivio, T. L., & Silhavy, T. J. (1999). The σ^{E} and Cpx regulatory pathways: Overlapping but distinct envelope stress responses. *Current Opinions in Microbiology*, *2*, 159–165.

Raymond, K. N., Dertz, E. A., & Kim, S. S. (2003). Enterobactin: An archetype for microbial iron transport. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 3584–3588.

Reissbrodt, R., Kingsley, R., Rabsch, W., Beer, W., Roberts, M., & Williams, P. H. (1997). Iron-regulated excretion of α-keto acids by *Salmonella typhimurium*. *Journal of Bacteriology*, *179*, 4538–4544.

Rezuchova, B., Miticka, H., Homerova, D., Roberts, M., & Kormanec, J. (2003). New members of the *Escherichia coli* σ^{E} regulon identied by a two-plasmid system. *FEMS Microbiology Letters*, *225*, 1–7.

Rhodius, V. A., Suh, W. C., Nonaka, G., West, J., & Gross, C. A. (2006). Conserved and variable functions of the σ^{E} stress response in related genomes. *PLoS Biology*, *4*, 0043–0059.

Roeßler, M., & Müller, V. (2001). Osmoadaptation in bacteria and archaea: Common principles and differences. *Environmental Microbiology*, *3*, 743–754.

Romeis, T., & Höltje, J. V. (1994). Penicillin-binding protein 7/8 of *Escherichia coli* is a DD-endopeptidase. *European Journal of Biochemistry*, *224*, 597–604.

Römling, U. (2005). Characterization of the rdar morphotype, a multicellular behaviour in *Enterobacteriaceae*. *Cellular and Molecular Life Sciences*, *62*, 1234–1246.

Roszak, D. B., Grimes, D. J., & Colwell, R. R. (1984). Viable but nonrecoverable stage of *Salmonella enteritidis* in aquatic systems. *Canadian Journal of Microbiology*, *30*, 334–338.

Rowley, G., Spector, M., Kormanec, J., & Roberts, M. (2006). Pushing the envelope: Extracytoplasmic stress responses in bacterial pathogens. *Nature Reviews Microbiology*, *4*, 383–394.

Rowley, G., Skovierova, H., Stevenson, A., Rezuchova, B., Homerova, D., Lewis, C., et al. (2010). The periplasmic chaperone Skp is required for successful *Salmonella* Typhimurium infection in a murine typhoid model. *Microbiology*, *157*, 848–858.

Ruiz, N., & Silhavy, T. J. (2005). Sensing external stress: Watchdogs of the *Escherichia coli* cell envelope. *Current Opinion in Microbiology*, *8*, 122–126.

Rychlik, I., & Barrow, P. A. (2005). *Salmonella* stress management and its relevance to behaviour during intestinal colonisation and infection. *FEMS Microbiology Reviews*, *29*, 1021–1040.

Seymour, R., Mishra, P. V., Khan, M. A., & Spector, M. P. (1996). Essential roles of starvation-stress response loci in carbon-starvation-inducible cross-resistance and hydrogen peroxide-inducible adaptive resistance to hydrogen peroxide challenge in *Salmonella typhimurium*. *Molecular Microbiology*, *20*, 497–505.

Sharma, U. K., & Chatterji, D. (2010). Transcriptional switching in *Escherichia coli* during stress and starvation by modulation of σ^{70} activity. *FEMS Microbiological Reviews*, *34*, 646–657.

Skaar, E. (2010). The battle for iron between bacterial pathogens and their vertebrate hosts. *PLoS Pathogens*, *6*, 1–4.

Skovierova, H., Rowley, G., Rezuchova, B., Homerova, D., Lewis, C., Roberts, M., et al. (2006). Identification of the σ^{E} regulon of *Salmonella enterica* serovar Typhimurium. *Microbiology*, *152*, 1347–1359.

Soncini, F. C., Garcia-Vescovi, E., Solomon, F., & Groisman, E. A. (1996). Molecular basis of the magnesium deprivation response in *Salmonella typhimurium*: Identification of PhoP-regulated genes. *Journal of Bacteriology*, *178*, 5092–5099.

Sonck, K. A., Kint, G., Schoofs, G., Vander Wauven, C., Vanderleyden, J., & De Keersmaecker, S. C. J. (2009). The proteome of *Salmonella* Typhimurium grown under *in vivo*-mimicking conditions. *Proteomics*, *9*, 565–579.

Spector, M. P. (1990). Gene expression in response to multiple nutrient-starvation conditions in *Salmonella typhimurium*. *FEMS Microbiology Ecology*, *74*, 175–184.

Spector, M. P. (1998). The starvation-stress response (SSR) of *Salmonella*. *Advances in Microbial Physiology*, *40*, 233–279.

Spector, M. P. (2010). Metabolism, central (intermediary). In M. Schaechter (Ed.), *The desk encyclopedia of microbiology* (pp. 728–750). (Second Edition). Oxford, U.K.: Academic Press.

Spector, M. P., & Cubitt, C. L. (1992). Starvation-inducible loci of *Salmonella typhimurium:* Regulation and roles in starvation survival. *Molecular Microbiology*, *6*, 1467–1476.

Spector, M. P., & Foster, J. W. (1993). Starvation-stress response (SSR) of *Salmonella typhimurium:* Gene expression and survival during nutrient starvation. In S. Kjelleberg (Ed.), *Starvation in bacteria* (pp. 201–224). New York: Plenum Publishing.

Spector, M. P., Aliabadi, Z., Gonzalez, T., & Foster, J. W. (1986). Global control in *Salmonella typhimurium:* Two-dimensional electrophoretic analysis of starvation-, anaerobiosis-, and heat shock-inducible proteins. *Journal of Bacteriology*, *168*, 420–424.

Spector, M. P., Park, Y. K., Tirgari, S., Gonzalez, T., & Foster, J. W. (1988). Identification and characterization of starvation-regulated genetic loci in *Salmonella typhimurium* by using Mud-directed *lacZ* operon fusions. *Journal of Bacteriology*, *170*, 345–351.

Spector, M. P., DiRusso, C. C., Pallen, M. J., Garcia del-Portillo, F., Dougan, G., & Finlay, B. B. (1999). The medium-/long-chain fatty acyl-CoA dehydrogenase (*fadF*) gene of *Salmonella typhimurium* is a phase 1 starvation-stress response (SSR) locus. *Microbiology*, *145*, 15–31.

Spector, M. P., Garcia del Portillo, F., Bearson, S. M., Mahmud, A., Magut, M., Finlay, B. B., et al. (1999). The *rpoS*-dependent starvation-stress response locus *stiA* encodes a nitrate reductase (*narZYWV*) required for carbon-starvation-inducible thermotolerance and acid tolerance in *Salmonella typhimurium*. *Microbiology*, *145*, 3035–3045.

Stephenson, K., & Lewis, R. J. (2005). Molecular insights into the initiation of sporulation in Gram-positive bacteria: New technologies for an old phenomenon. *FEMS Microbiological Reviews*, *29*, 281–301.

Stevens, M. P., Humphrey, T. J., & Maskell, D. J. (2009). Molecular insights into farm animal and zoonotic *Salmonella* infections. *Philosophical Transactions of the Royal Society B*, *364*, 2709–2723.

Stocker, B. A. D., & Makela, P. H. (1986). Genetic determinance of bacterial virulence with special reference to *Salmonella*. *Microbiology and Immunology*, *124*, 149–172.

Storz, G., & Imlay, J. A. (1999). Oxidative stress. *Current Opinions in Microbiology*, *2*, 188–194.

Storz, G., & Zheng, M. (2000). Oxidative stress. In G. Storz, & R. Hengge-Aronis (Eds.), *Bacterial stress responses* (pp. 47–59). Washington, D.C.: ASM Press.

Storz, G., Tartaglia, L. A., & Ames, B. N. (1990a). The OxyR regulon. *Antonie Van Leeuwenhoek*, *58*, 157–161.

Storz, G., Tartaglia, L. A., & Ames, B. N. (1990b). Transcriptional regulator of oxidative stress-inducible genes: Direct activation by oxidation. *Science*, *248*, 189–194.

Straus, D. B., Walter, W. A., & Gross, C. A. (1987). The heat shock response of *E. coli* is regulated by changes in the concentration of σ^{32}. *Nature*, *329*, 348–351.

Strøm, A. R., & Kaasen, I. (1993). Trehalose metabolism in *Escherichia coli*: Stress protection and stress regulation of gene expression. *Molecular Microbiology*, *8*, 205–210.

Sukharev, S. I., Blount, P., Martinac, B., & Kung, C. (1997). Mechanosensitive channels of *Escherichia coli*: The MscL gene, protein, and activities. *Annual Review of Physiology*, *59*, 633–657.

Sydenham, M., Douce, G., Bowe, F., Ahmed, S., Chatfield, S., & Dougan, G. (2000). *Salmonella enterica* serovar Typhimurium *surA* mutants are attenuated and effective live oral vaccines. *Infection and Immunity*, *68*, 1109–1115.

Takaya, A., Suzuki, M., Matsui, H., Tomoyasu, T., Sashinami, H., Nakane, A., et al. (2003). Lon, a stress-induced ATP-dependent protease, is critically important for systemic *Salmonella enterica* serovar Typhimurium infection of mice. *Infection and Immunity*, *71*, 690–696.

Takaya, A., Tomoyasu, T., Matsui, H., & Yamamoto, T. (2004). The DnaK/DnaJ chaperone machinery of *Salmonella enterica* serovar Typhimurium is essential for invasion of epithelial cells and survival within macrophages, leading to systemic infection. *Infection and Immunity*, *72*, 1364–1373.

Tamaru, Y., Takani, Y., Yoshida, T., & Sakamoto, T. (2005). Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. *Applied and Environmental Microbiology*, *71*, 7327–7333.

Taylor, P. D., Inchley, C. J., & Gallagher, M. P. (1998). The *Salmonella typhimurium* AhpC polypeptide is not essential for virulence in BALB/c mice but is recognized as an antigen during infection. *Infection and Immunity*, *66*, 3208–3217.

Testerman, T. L., Vazquez-Torres, A., Xu, Y., Jones-Carson, J., Libby, S. J., & Fang, F. C. (2002). The alternative sigma factor σ^{E} controls antioxidant defenses required for *Salmonella* virulence and stationary-phase survival. *Molecular Microbiology*, *43*, 771–782.

Thede, G. L., Arthur, D. C., Edwards, R. A., Buelow, D. R., Wong, J. L., Raivio, T. L., et al. (2011). Structure of the periplasmic stress response protein CpxP. *Journal of Bacteriology*, *193*, 2149–2157.

Thomsen, L. E., Olsen, J. E., Foster, J. W., & Ingmer, H. (2002). ClpP is involved in the stress response and degradation of misfolded proteins in *Salmonella enterica* serovar Typhimurium. *Microbiology*, *148*, 2727–2733.

Tomoyasu, T., Gamer, J., Bukau, B., Kanemori, M., Mori, H., Rutman, A. J., et al. (1995). *Escherichia coli* FtsH is a membrane-bound, ATP-dependent protease, which degrades the heat-shock transcription factor σ^{32}. *EMBO Journal*, *14*, 2551–2560.

Tomoyasu, T., Ogura, T., Tatsuta, T., & Bukau, B. (1998). Levels of DnaK and DnaJ provide tight control of heat shock gene expression and protein repair in *Escherichia coli*. *Molecular Microbiology*, *30*, 567–581.

Touati, D. (2000a). Iron and oxidative stress in bacteria. *Archives of Biochemistry and Biophysics*, *373*, 1–6.

Touati, D. (2000b). Sensing and protecting against superoxide stress in *Escherichia coli* — How many ways are there to trigger *soxRS* response? *Redox Report*, *5*, 287–293.

Touati, D., Jacques, M., Tardat, B., Bouchard, L., & Despied, S. (2000). Lethal oxidative damage and mutagenesis are generated by iron in delta *fur* mutants of *Escherichia coli*: Protective role of superoxide dismutase. *Journal of Bacteriology*, *177*, 2305–2314.

Tsaneva, I. R., & Weiss, B. (1990). *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. *Journal of Bacteriology*, *172*, 4197–4205.

Tsolis, R. M., Bäumler, A. J., Stojiljkovic, I., & Heffron, F. (1995). Fur regulon of *Salmonella typhimurium*: Identification of new iron-regulated genes. *Journal of Bacteriology*, *177*, 4628–4637.

Tsoy, O., Ravcheev, D., & Mushegian, A. (2009). Comparative genomics of ethanolamine utilization. *Journal of Bacteriology*, *191*, 7157–7164.

Tuomanen, E., & Schwartz, J. (1987). Penicillin-binding protein 7 and its relationship to lysis of non-growing *Escherichia coli*. *Journal of Bacteriology*, *169*, 4912–4915.

Turner, J. M., & Messenger, A. J. (1986). Occurrence, biochemistry and physiology of phenazine pigment production. *Advances in Microbial Physiology*, *27*, 211–275.

Turpin, P. E., Maycroft, K. A., Rowlands, C. L., & Wellington, E. M. H. (1993). Viable but nonculturable salmonellas in soil. *Journal of Applied Bacteriology*, *74*, 421–427.

Typas, A., Becker, G., & Hengge, R. (2007). The molecular basis of selective promoter activation by the σ^{S} subunit of RNA polymerase. *Molecular Microbiology*, *63*, 1296–1306.

Uzzau, S., Bossi, L., & Figuerosa-Bossi, N. (2002). Differential accumulation of *Salmonella* [Cu, Zn] superoxide dismutases SodCI and SodCII in intracellular bacteria: Correlation with their relative contribution to pathogenicity. *Molecular Microbiology*, *46*, 147–156.

VanBogelen, R. A., Kelley, P. M., & Neidhardt, F. C. (1987). Differential induction of heat shock, SOS and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli*. *Journal of Bacteriology*, *169*, 26–32.

Van Velkinburgh, J. C., & Gunn, J. S. (1999). PhoP-PhoQ-regulated loci are required for enhanced bile resistance in *Salmonella* spp. *Infection and Immunity*, *67*, 1614–1622.

Velayudhan, J., Castor, M., Richardson, A., Main-Hester, K. L., & Fang, F. C. (2007). The role of ferritins in the physiology of *Salmonella enterica* sv. Typhimurium: A unique role for ferritin B in iron-sulphur cluster repair and virulence. *Molecular Microbiology*, *63*, 1495–1507.

Vestby, L. K., Møretrø, T., Ballance, S., Langsrud, S., & Nesse, L. L. (2009). Survival potential of wild type cellulose deficient *Salmonella* from the feed industry. *BMC Veterinary Research*, *5*, 43.

Wandersman, C., & Delepelaire, P. (2004). Bacterial iron sources: From siderophores to hemophores. *Annual Review of Microbiology*, *58*, 611–647.

Walkup, L. K. B., & Kogoma, T. (1989). *Escherichia coli* proteins inducible by oxidative stress mediated by the superoxide radical. *Journal of Bacteriology*, *171*, 1476–1484.

Walter, D., Ailion, M., & Roth, J. (1997). Genetic characterization of the *pdu* operon: Use of 1, 2-propanediol in *Salmonella* Typhimurium. *Journal of Bacteriology*, *179*, 1013–1022.

Wang, Q., & Kaguni, J. M. (1989). A novel sigma factor is involved in expression of the *rpoH* gene of *Escherichia coli*. *Journal of Bacteriology*, *171*, 4248–4253.

Weatherspoon-Griffin, N., Zhao, G., Kong, W., Kong, Y., Morigen, Andrews-Polymenis H., McClelland, M., et al. (2011). The CpxR/CpxA two-component system up-regulates two Tat-dependent peptidoglycan amidases to confer bacterial resistance to antimicrobial peptide. *Journal of Biological Chemistry*, *286*, 5529–5539.

Webb, C., Moreno, M., Wilmes-Riesenberg, M., Curtiss, R., III, & Foster, J. W. (1999). Effects of DksA and ClpP protease on sigma S production and virulence in *Salmonella typhimurium*. *Molecular Microbiology*, *34*, 112–123.

Weiner, L., & Model, P. (1994). Role of an *Escherichia coli* stress–response operon in stationary-phase survival. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 2191–2195.

White, A. P., & Surette, M. G. (2006). Comparative genetics of the rdar morphotype in *Salmonella*. *Journal of Bacteriology*, *188*, 8395–8406.

White, A. P., Gibson, D. L., Kim, W., Kay, W. W., & Surette, M. G. (2006). Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of *Salmonella*. *Journal of Bacteriology*, *188*, 3219–3227.

Williams, P. H., Rabsch, W., Methner, U., Voigt, W., Tschäpe, H., & Reissbrodt, R. (2006). Catecholate receptor proteins in *Salmonella enterica*: role in virulence and implications for vaccine development. *Vaccine*, *24*, 3840–3844.

Winfield, M. D., & Groisman, E. A. (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology*, *69*, 3687–3694.

Wood, J. M. (2006). Osmosensing by bacteria. *Science Signalling Transduction Knowledge Environment*, *357*, 43.

Wood, J. M. (2007). Bacterial osmosensing transporters. *Methods in Enzymology*, *428*, 77–107.

Wood, J. M., Bremer, E., Csonka, L. N., Kraemer, R., Poolman, B., van der Heide, T., et al. (2001). Osmosensing and osmoregulatory compatible solute accumulation by bacteria. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *130*, 437–460.

Wosten, M. M., Kox, L. F., Chamnongpol, S., Soncini, F. C., & Groisman, E. A. (2000). A signal transduction system that responds to extracellular iron. *Cell*, *103*, 113–125.

Wu, J., & Weiss, B. (1991). Two divergently transcribed genes, *soxR* and *soxS*, control a superoxide response regulon of *Escherichia coli*. *Journal of Bacteriology*, *173*, 2864–2871.

Xu, J., & Johnson, R. C. (1995). *aldB*, an RpoS-dependent gene in *Escherichia coli* encoding an aldehyde dehydrogenase that is repressed by Fis and activated by CRP. *Journal of Bacteriology*, *177*, 3166–3175.

Xu, H., Lee, H. Y., & Ahn, J. (2008). Cross-protective effect of acid-adapted *Salmonella enterica* on resistance to lethal acid and cold stress conditions. *Letters in Applied Microbiology*, *47*, 290–297.

Yura, T., & Nakahigashi, K. (1999). Regulation of the heat-shock response. *Current Opinions in Microbiology*, *2*, 153–158.

Yura, T., Kanemori, M., & Morita, T. (2000). The heat shock response: Regulation and function. In G. Storz, & R. Hengge-Aronis (Eds.), *Bacterial stress responses* (pp. 3–18). Washington, D.C.: ASM Press.

Zhang, A., Altuvia, S., Tiwari, A., Argaman, L., Hengge-Aronis, R., & Storz, G. (1998). The OxyS regulatory RNA represses *rpoS* translation and binds the Hfq (HF-I) protein. *EMBO Journal*, *17*, 6061–6068.

Zheng, M., Åslund, F., & Storz, G. (1998). Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science*, *279*, 1718–1721.

Zheng, M., Doan, B., Schneider, T. D., & Storz, G. (1999). OxyR and SoxRS regulation of *fur*. *Journal of Bacteriology*, *181*, 4639–4643.