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Review

Bacterial Stress Responses during Host Infection

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Pathogenic bacteria must withstand diverse host environments during infection. Environmental signals, such as pH, temperature, nutrient limitation, etc., not only trigger adaptive responses within bacteria to these specific stress conditions but also direct the expression of virulence genes at an appropriate time and place. An appreciation of stress responses and their regulation is therefore essential for an understanding of bacterial pathogenesis. This review considers specific stresses in the host environment and their relevance to pathogenesis, with a particular focus on the enteric pathogen *Salmonella*.

Stresses in the Host Environment

Bacteria experience stress from their initial moment of contact with the host. For most pathogens, this entails a change in temperature. For bacteria transmitted by arthropod vectors, this also involves a transition from the insect gut to mammalian subcutaneous tissue or the bloodstream (Hinnebusch, 2005). Respiratory pathogens must cope with an array of host-derived antimicrobial mediators, including bactericidal peptides produced by epithelial cells (Grubor et al., 2006), and may also be required to adapt to nitrosative stress (Lundberg, 2008), hyperosmolarity (Henderson et al., 2014), and oxygen limitation (Worlitzsch et al., 2002). In contrast, enteric pathogens are ingested and must survive the hostile environment of the stomach, which is notable for a strongly acidic pH (Foster, 1999) and the presence of reactive nitrogen species generated from dietary nitrate (Duncan et al., 1995) (Figure 1). Within the intestinal lumen, enteric pathogens encounter membrane-active antimicrobial peptides (Ouellette, 2011), bile salts, free fatty acids, enhanced osmolarity, and changing oxygen tensions (Guiney, 1997). Host inflammatory responses recruit phagocytic cells, subjecting pathogens to oxidative and nitrosative stress (Fang, 2004). Host sequestration of essential metals and other nutrients creates additional challenges as intracellular pathogens such as *Francisella* and *Salmonella* respond to distinctive cytoplasmic or phagosomal environments, respectively (Steele et al., 2013; Nairz et al., 2015).

To survive these changing environments, bacteria have developed exquisite systems that not only sense these stresses but also trigger appropriate responses that allow survival and propagation under these conditions. This review examines the various stresses encountered by pathogenic bacteria during infection and the mechanisms by which they sense and respond to host conditions.

Acid pH

Pathogenic bacteria encounter acid pH in the gastrointestinal and genital tracts, skin, and endocytic vesicles of the intracellular degradative pathway. To adapt to acid pH, many pathogenic bacteria express two-component systems (TCSs), such as the well-characterized PmrB/PmrA, PhoQ/PhoP, EvgS/EvgA, and EnvZ/OmpR systems, that sense environmental acidic pH and then initiate a signaling cascade allowing adaptation to these conditions. The transcriptional repressor Fur (ferric uptake regulator) and the alternative sigma factors σ^S and σ^E, which control gene expression via interactions with the bacterial RNA polymerase, also have established roles in altering bacterial gene expression to respond to pH-induced stress. Several of these regulators, in particular PhoP, OmpR, and σ^S, also control the expression of genes required for *Salmonella* virulence (Table 1) (Fang et al., 1992; Belden and Miller, 1994; Lee et al., 2000). Additionally, ionizable imidazole and carboxylic groups in His, Asp, and Glu residues endow some transcriptional regulators such as ArsS, CadC, EvgS, PhoQ, PmrB, and SsrA with the capacity to sense acid pH (Haneburger et al., 2011; Mulder et al., 2015; Müller et al., 2009; Perez and Groisman, 2007; Prost et al., 2007) (Figure 2A). Some of the adaptive responses to acid pH initiated by the PhoQ/PhoP and EvgS/EvgA TCS work via transcriptional activation of the *iraP* and *iraM* genes encoding anti-adaptor proteins, which outcompete σ^S for binding to the response regulatory RssB, thus preventing RssB-triggered σ^S degradation by the ClpXP protease (Bearson et al., 1996; Eguchi et al., 2011; Tu et al., 2006). Expression of various decarboxylases and genes involved in central metabolism, transport, and membrane composition allows the cell to adapt to acidic conditions.

The biological relevance of acid stress to pathogenesis has been clearly shown. Both phosphorylation-dependent and phosphorylation-independent signaling by the ArsR/ArsS TCS allows *Helicobacter pylori* to withstand the acidic conditions of the stomach (Marcus et al., 2016). The primary function of ArsR/ArsS is to regulate the production and trafficking of urease, which allows urea-dependent buffering of gastric acid. Acidification of the macrophage phagosome provides an essential signal for activation of *Salmonella* virulence genes required for intracellular survival and growth, and blocking phagosomal acidification paradoxically attenuates *Salmonella* virulence (Rathman et al., 1996). Acid-induced activation of the TCS response regulator OmpR leads to expression of the *Salmonella* pathogenicity island-2 (SPI2) type III secretion system (T3SS) (Chakraborty

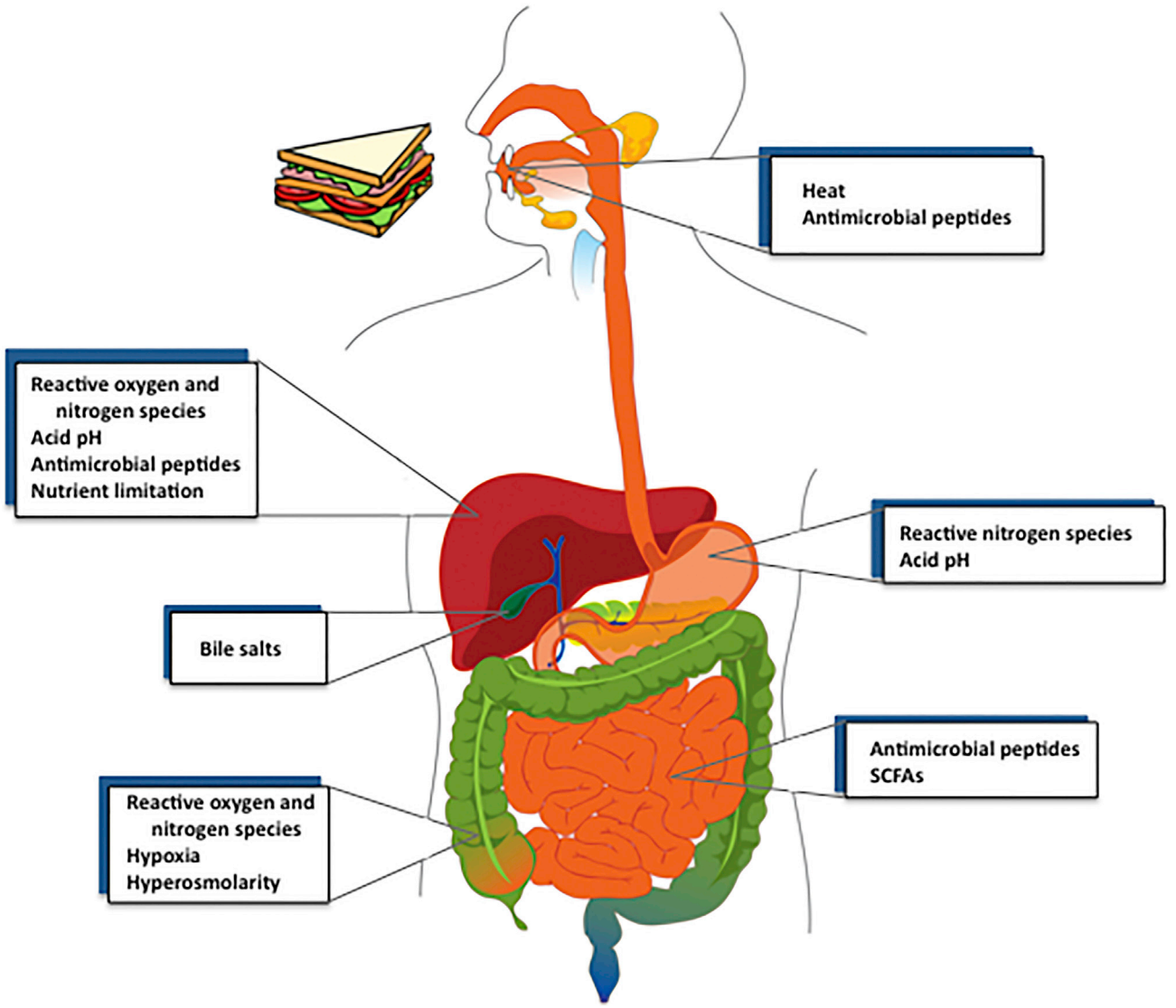

Figure 1. Stresses Encountered by a Typical Foodborne Enteric Pathogen
Following ingestion, an enteric pathogen encounters distinctive stress conditions in the oral cavity, stomach, small intestine, and colon of the host. Invasive pathogens must also withstand stresses imposed by phagocytic cells following the invasion of deeper tissues and the bloodstream (modified from a drawing by Mariana Ruiz Villarreal, Wikimedia Commons).

et al., 2015), which delivers effector proteins to facilitate infection and also promotes activation of the acid-sensing PhoP/PhoQ regulon (Alpuche Aranda et al., 1992; Martin-Orozco et al., 2006), although pH sensing by PhoQ appears to be dispensable for *Salmonella* virulence (Hicks et al., 2015).

Oxidative and Nitrosative Stress

Flavoproteins and quinones in the electron transport chain engender endogenous oxidative stress via the adventitious univalent reduction of molecular oxygen (O_2) to superoxide ($O_2^{\cdot-}$). However, the low levels of oxidative stress generated in these metabolic processes are dwarfed by the micromolar amounts of oxyradicals synthesized by the NADPH oxidase during the respiratory burst of professional phagocytes that serves to damage biomolecules of invading microbes (Fang, 2004), and it is important to distinguish between bacterial defenses designed to respond to low or high levels of oxidative stress (Fang, 2011). Bacteria may be exposed to nitric oxide ($NO\cdot$) and reactive nitrogen species (RNS) such as S-nitrosothiols and peroxynitrite ($ONOO^-$) generated in reactions of $NO\cdot$ with O_2, $O_2^{\cdot-}$, organic and inorganic radicals, iron, or low-molecular-weight thiols. $NO\cdot$ is produced enzymatically by microbial nitrate and nitrite reductases as well as by bacterial and host cell $NO\cdot$ synthases.

Diverse bacterial regulatory proteins, including OxyR, DksA, SsrB, OhrR, MosR, SarZ, and MgtA, exploit the redox properties of Cys residues to sense hydrogen peroxide (H_2O_2), *S*-nitrosothiols (RSNO), or $ONOO^-$ (Vázquez-Torres, 2012). Sulfenic acid (RSOH), disulfides (RSSR), and *S*-nitrosothiols are some of the most abundant products arising from oxidative and nitrosative modifications of thiol groups (Geiger et al., 2010). In addition to

Table 1. Examples of Stress Regulators Involved in Virulence and Virulence Gene Expression

Regulator	Stress Response	Role in Virulence	Reference
ArsA	acid pH	required for *H. pylori* gastric survival and virulence	Marcus et al., 2016
BvgA	temperature	regulates toxin and T3SS expression in *B. pertussis*	Prugnola et al., 1995
CsrA	starvation	regulates VirB and VirF expression in *S. flexneri*	Gore and Payne, 2010
		regulates SPI-1 and SPI-2 in *S. enterica* via SirA and HilD	Martínez et al., 2011
DosR	hypoxia/NO·	regulates dormancy in *M. tuberculosis*	Converse et al., 2009
DtxR	iron deprivation	regulates toxin production in *C. diphtheriae*	Boyd et al., 1990
FNR	hypoxia	regulates expression of fimbriae, hemolysin, and pathogenicity island genes in uropathogenic *E. coli*	Barbieri et al., 2014
		regulates T3SS expression and effector secretion in *S. flexneri*	Marteyn et al., 2010
H-NS	temperature	regulates expression of *S. flexneri* VirF, *E. coli* hemolysin, and *S.* Typhimurium SPI-2 T3SS	Falconi et al., 1998; Madrid et al., 2002
LexA	DNA damage	regulates Shiga toxin in EHEC and cholera toxin in *V. cholerae*	Waldor and Friedman, 2005
NsrR	NO·	regulates genes required for NO· resistance and virulence of *S. enterica*	Karlinsey et al., 2012
OmpR	acid pH/osmotic stress	regulates SPI-1 and SPI-2 gene expression in *S. enterica* via HilC and SsrA (SpiR)	Chakraborty et al., 2015; Lee et al., 2000
OxyR	oxidative stress	required for *S. enterica* virulence in chickens, swine, and cattle	Taylor et al., 1998; Chaudhuri et al., 2013
PhoP	antimicrobial peptides	regulates pathogenicity island gene expression and other genes required for *S. enterica* virulence	Belden and Miller, 1994
σ^E	misfolded OMPs	required for *S. enterica* virulence and survival in macrophages	Humphreys et al., 1999
	acid pH		
σ^S	starvation	regulates expression of the *S. enterica spv* plasmid virulence genes	Fang et al., 1992
SrrA (ResD)	hypoxia/NO·	regulates genes required for NO· resistance and anaerobic metabolism in *S. aureus*	Kinkel et al., 2013

Cys residues, iron is frequently used by regulatory proteins to sense O_2, NO·, carbon monoxide (CO), $O_2^{\cdot-}$, or redox cycling compounds. Iron can be found as (1) mononuclear centers in Fur, PerR (peroxide regulator), or NorR (NO· regulator); (2) [2Fe-2S] and [4Fe-4S] clusters in SoxR (superoxide regulator), FNR (fumarate-nitrate reduction regulator), or NsrR (NO·-responsive regulator); or (3) heme prosthetic groups in dormancy survival regulatory proteins DosT and DosS, or DNR (dissimilative nitrate respiration regulator) (Crack et al., 2014; Ji et al., 2015; Lobato et al., 2014; Ma et al., 2012). Molecular mechanisms of redox sensing rely on protein conformational changes triggered by interaction with reactive oxygen and nitrogen species (see accompanying review in this issue; Fang et al., 2016) (Figure 2B). Expression of antioxidant enzymes (catalase, superoxide dismutase) or NO·-detoxifying proteins (NO· reductase, flavohemoglobin), as well as stress-resistant enzymes and cluster repair machinery, enhances resistance to oxidative or nitrosative stress.

Responsiveness to different concentrations of reactive oxygen or nitrogen species can distinguish between adaptive responses and consequences of stress. Higher concentrations of NO· are required to activate Fur, NorR, or SoxR in comparison to NsrR responses (Karlinsey et al., 2012), consistent with the primary importance of NsrR-regulated gene products in NO· detoxification. Some sensors of oxidative and nitrosative stress do not respond directly to O_2 or NO·, but rather sense secondary redox changes. For example, the ArcB/ArcA, SrrA/SrrB, and ResD/ResE TCSs of Enterobacteriaceae, *Staphylococcus aureus*, and *Bacillus subtilis* respond to the redox state of the quinone pool, which varies with O_2 availability or the presence of NO· (Nakano, 2002; Durand et al., 2015; Georgellis et al., 2001; Kinkel et al., 2013).

Envelope Stress

Common sources of cell envelope stress include antimicrobial cationic peptides, bile, acid pH, misfolded secretins and outer membrane proteins, alterations in phospholipids and lipopolysaccharides, and changes in proton motive force (PMF). The aforementioned PhoP/PhoQ TCS responds to antimicrobial peptides (Bader et al., 2005) and promotes cell envelope alterations that confer peptide resistance (Miller et al., 1990). The Bae, Cpx, and Rcs TCSs; phage shock proteins (Psps); and the alternative sigma factor σ^E regulate the envelope stress response (Raivio, 2005). Although these regulators control the expression of largely discrete subsets of genes, they appear to have complementary actions, as the elimination of one envelope stress pathway often results in enhanced expression of another (Humphreys et al., 2004; Becker et al., 2005). Control of the envelope stress response is perhaps best characterized with regard to σ^E and Psp. The extracytoplasmic sigma factor σ^E is tethered to the cytoplasmic membrane by the anti-sigma factor RseA (Figure 2C). Release of σ^E from RseA is a two-step process initiated upon activation of the DegS type 1 protease (T1P). This initial proteolytic event triggers complete degradation of RseA by the T2P protease RseP, thus releasing σ^E to

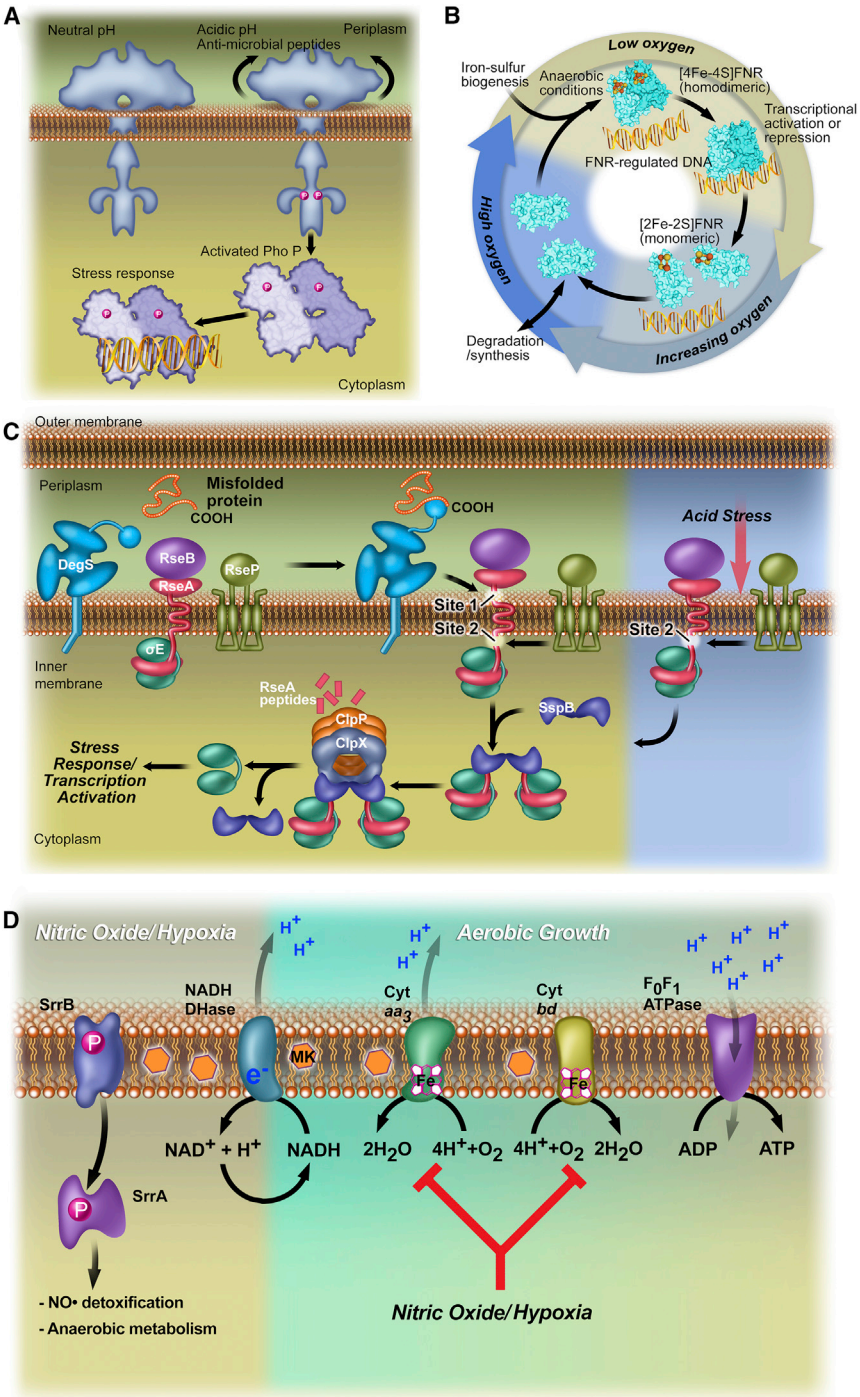

Figure 2. Examples of Stress-Responsive Regulatory Systems

(A) PhoPQ two-component system. A conformational change in the PhoQ periplasmic sensor domain under acidic conditions or upon binding by cationic pepdides leads to a conformational change and activation of cytoplasmic domain kinase activity. PhoQ autophosphorylation followed by phosphorylation of the cognate response regulator PhoP results in the activation of stress-response genes.

(B) FNR transcriptional regulator. Under anaerobic conditions, the transcriptional regulator FNR exists as a DNA-binding homodimer containing a [4Fe-4S] cluster. At increasing oxygen levels, degradation of the cluster produces inactive [2Fe-2S] cluster-containing FNR monomers that further degrade to a monomeric apo-protein.

(C) σ^E regulatory cascade. Misfolded proteins in the periplasm interact with the DegS protease to relieve PDZ-mediated inhibition and allow cleavage of the anti-sigma RseA at site 1. This in turn allows RseP to cleave RseA at site 2, releasing the alternative sigma factor σ^E into the cytoplasm. The adaptor protein SspB directs the σ^E-RseA complex to the ClpX/P protease, releasing σ^E to activate stress gene expression. Alternatively, acidic conditions allow RseA cleavage by RseP at site 2 (red arrow) in the absence of initial site 1 processing by DegS.

(D) SrrAB two-component system. Under aerobic conditions, electrons are carried by menaquinone (MK, orange) from the NADH dehydrogenase (blue) to the Cyt *aa3* and Cyt *bd* terminal oxidases (green and yellow). During hypoxia or following binding of the oxidase heme centers by NO·, reduction of the menaquinone pool activates the sensor kinase SrrB, which in turn phosphorylates the response regulator SrrA, leading to the activation of genes involved in anaerobic metabolism and NO· detoxification.

alter gene expression that facilitates adaptation to this membrane stress.

The Psp stress response monitors the state of the PMF. Inducing signals such as unfolded proteins trigger partner switching within members of the Psp family, such as the switch in binding of membrane-bound PspC from PspB to PspA (Flores-Kim and Darwin, 2015). Trafficking of PspA to the membrane prevents proton leakage and maintains PMF (Brissette et al., 1990), while releasing PspF in the cytoplasm induces activation of σ^{54}-dependent transcription of the *pspABCDE* operon (Darwin, 2013). The maintenance of PMF is expected to influence diverse processes important for pathogenesis, including bacterial motility, resistance to host-derived antimicrobial mediators, secretion, and nutrient acquisition (Karlinsey et al., 2010).

DNA Damage

Reactive oxygen and nitrogen species can directly damage DNA bases or indirectly promote DNA damage by interfering with DNA replication (Imlay and Linn, 1988; Schapiro et al., 2003; Richardson et al., 2009). Extreme acid pH can also damage DNA (Jeong et al., 2008). DNA damage is sensed in many bacterial species by the RecA protein, which binds single-stranded DNA and promotes autoproteolysis of the LexA transcriptional repressor to activate an adaptive program known as the "SOS response" (Shinagawa, 1996). The SOS response temporarily arrests cell division (Huisman et al., 1984) and activates pathways to bypass or repair damaged DNA. *Salmonella* mutants deficient in DNA repair are more sensitive to oxidative and nitrosative stress

and exhibit reduced virulence (Buchmeier et al., 1993, 1995; Schapiro et al., 2003). LexA also regulates the expression of genes carried by lambdoid phages, including virulence factors such as Shiga and cholera toxin (Waldor and Friedman, 2005). Thus, activation of the DNA damage response can also induce virulence gene expression.

Nutrient Limitation

In the host environment, access to carbohydrates, amino acids, and metals varies widely. The mammalian gut can be relatively rich in certain nutrients while intracellular environments are more restricted. Nutrient availability is further modulated by the activities of the host microbiota and host proteins.

Carbohydrate availability varies throughout the gut due to the breakdown of complex carbohydrates by commensal *Lactobacilli* and *Bacteroidetes*. Some of these breakdown products, such as short-chain fatty acids and glucose, serve as signals for pathogenic bacteria to induce virulence gene expression. The FruR/Cra regulator commonly found in enteric bacterial pathogens senses whether available carbohydrate sources can promote glycolysis or gluconeogenesis by interacting with fructose-1-phosphate and, to a lesser extent, fructose-1,6-bisphosphate (Ramseier et al., 1993). In addition to directing carbon flow appropriately, FruR/Cra also regulates virulence gene expression in *Salmonella*, *Shigella flexneri*, and enterohemorrhagic *E. coli* (EHEC) (Yoon et al., 2009; Gore and Payne, 2010; Njoroge et al., 2012). The CsrA regulator acts oppositely of FruR/Cra, inducing genes involved in glycolysis while repressing those involved in gluconeogenesis. CsrA is required for *S. flexneri* attachment and invasion and is involved in the expression of virulence genes in *Salmonella* through effects on the TCS BarA/SirA and transcriptional regulator HilD, which control the activation of invasion genes (Gore and Payne, 2010; Martínez et al., 2011). In *Yersinia*, the cAMP receptor protein Crp also contributes to the regulation of Csr and virulence gene expression (Heroven et al., 2012).

The stringent response to amino acid starvation is mediated by the small molecule guanosine tetra/pentaphosphate ((p)ppGpp), synthesized by RelA from GDP or GTP following interactions with ribosomes that are stalled as a result of amino acid starvation (Haseltine and Block, 1973; Agirrezabala et al., 2013). (p)ppGpp interacts with RNA polymerase and a variety of proteins to promote the expression of virulence genes in *Francisella tularensis*, *Vibrio cholerae*, *Burkholderia pseudomallei*, *S. enterica*, *S. aureus*, *Y. pestis*, and EHEC, among others (Charity et al., 2009; Pal et al., 2012; Müller et al., 2012; Geiger et al., 2010; Sun et al., 2009; Nakanishi et al., 2006). Increased (p)ppGpp levels are also linked to increased σ^S levels through expression of the anti-adaptor proteins IraP and IraD, which block σ^S degradation (Bougdour and Gottesman, 2007; Merrikh et al., 2009). σ^S responds to a variety of starvation conditions and activates genes required for virulence in *Salmonella* (Fang et al., 1992). The stringent response is also regulated by the RNA polymerase-binding protein DksA, which regulates amino acid biosynthetic genes as well as antioxidant and antinitrosative defenses essential for *Salmonella* pathogenesis (Henard and Vázquez-Torres, 2012; Henard et al., 2014). In Gram-positive bacteria, CodY is involved in regulating virulence gene expression in response to amino acid availability (Geiger et al., 2010; Lobel et al., 2015). In *Listeria*, CodY induces expression of the virulence regulator PrfA when concentrations of branched-chain amino acids are low, while its role in *S. aureus* virulence gene regulation is more complex.

Perhaps one of the most important types of nutrient limitation in the host is metal limitation. Sequestration of metals has been termed "nutritional immunity," since availability of metals such as iron and zinc has significant effects on the ability of pathogens to replicate (Becker and Skaar, 2014). Metals are cofactors of proteins and enzymes involved in a variety of essential processes ranging from metabolism to DNA replication. Metal availability is sensed by metal-binding transcription factors that regulate the expression of genes involved in metal acquisition. The Fur transcriptional repressor controls genes that contribute to *Salmonella* survival and growth within the host (Troxell et al., 2011). Transcriptional repression by Fur is relieved when iron is limited, leading to the expression of iron acquisition systems, including those that produce and secrete iron-chelating siderophores such as enterobactin and salmochelin (Ernst et al., 1978; Bagg and Neilands, 1987; Hantke et al., 2003). A related regulator, Zur, controls the high-affinity zinc acquisition system ZnuABC in analogous fashion (Patzer and Hantke, 2000). Manganese acquisition, which protects cells from oxidative damage, is regulated by both the manganese-binding transcriptional regulator MntR and the oxidative stress regulator OxyR (Anjem et al., 2009). Manganese-bound MntR represses the expression of manganese acquisition systems in both Gram-negative and Gram-positive bacteria (Waters et al., 2011; Lieser et al., 2003). A related regulator, DtxR, binds iron to repress toxin production in *Corynebacterium diptheriae*, the causative agent of diphtheria, and regulates genes involved in iron homeostasis as well (Boyd et al., 1990; Yellaboina et al., 2004). A second iron-responsive DtxR family member called IdeR is important for the regulation of iron homeostasis in *M. tuberculosis* as well as for virulence (Pandey and Rodriguez, 2014).

Oxygen Limitation

Upon shifting from the external aerobic environment to the host, many pathogens encounter oxygen-limited conditions and adapt their metabolic pathways accordingly. SrrA/SrrB is a TCS in *S. aureus* that is similar to the ResD/ResE system of *B. subtilis*. In addition to regulating the *hmp* flavohemoglobin involved in NO· resistance, these systems also regulate genes required for survival under hypoxic conditions (Kinkel et al., 2013; Durand et al., 2015) (Figure 2D). ResD/ResE and SrrA/SrrB promote expression of the small RNA RsaE (RoxS) to repress genes involved in oxidative stress and redox reactions, thereby minimizing non-essential gene expression when electron transport is limited (Durand et al., 2015). Like ArcB/ArcA in *E. coli*, SrrA/SrrB and ResD/ResE appear to sense cellular respiration by sensing the redox status of the quinone pool. ArcB is a sensor kinase with two redox-active cysteine residues that are reduced by menaquinone, leading to autophosphorylation, phosphotransfer to the ArcA response regulator, and expression of genes required for anaerobic metabolism (Alvarez et al., 2013). Upon a return to aerobic conditions, the cysteine residues are oxidized by ubiquinone to deactivate ArcA. ArcB/ArcA also regulates conjugative transfer of the *Salmonella* pSLT virulence plasmid under microaerobic conditions by activating the *traY* promoter that controls the transfer operon (Serna et al., 2010). Interestingly, while ArcA in *S.* Typhimurium has

been shown to regulate both metabolic and flagellar genes, some genes involved in virulence are repressed by ArcA, while others appear to be activated. An *arcA* mutant has no virulence defect in mice (Lu et al., 2002), which may be explained by the relatively small number of virulence genes regulated by ArcA, as well as by co-regulation by other proteins such as FNR (Evans et al., 2011). In *M. tuberculosis*, the ability to sense and respond to hypoxia and nitrosative stress is important for intracellular survival (Converse et al., 2009) and implicated in latency (Boon and Dick, 2012). The adaptive response to these conditions is controlled by the DosR/DosS TCS, with a heme-containing GAF (cGMP-specific phophodiesterases, adenylyl cyclases, and FhlA) domain in DosS responsible for O_2/NO· sensing (Sardiwal et al., 2005).

The *E. coli* FNR protein is the model oxygen-sensing transcription factor responsible for responding to a shift from aerobic to anaerobic metabolism in facultative anaerobes. FNR and FNR-like regulators sense oxygen availability using a [4Fe-4S] cluster that is converted to the apo form of the protein via a [2Fe-2S] cluster intermediate in the presence of oxygen (Crack et al., 2004; Reinhart et al., 2008) (Figure 2B). The [4Fe-4S] form of the protein produces a stable homodimer capable of binding DNA to activate the expression of genes involved in anaerobic metabolism while repressing those involved in aerobic processes (Lazazzera et al., 1996). Regulation by FNR in *E. coli* appears to be more complicated than originally anticipated, since binding of FNR to a promoter is influenced by proteins such as the histone-like protein H-NS and does not always directly lead to transcriptional activation (Myers et al., 2013). Nevertheless, FNR has been shown to be important for *Salmonella* virulence and survival in macrophages (Fink et al., 2007). FNR is also important for the adherence, invasiveness, and virulence of uropathogenic *E. coli* (UPEC) (Barbieri et al., 2014) and *Shigella*, in which FNR prevents full T3SS activation and secretion of Ipa (invasion plasmid antigen) effectors in the anaerobic intestinal lumen that modify host processes to facilitate infection (Marteyn et al., 2010). T3SS repression is only relieved once cells encounter the zone of higher oxygenation immediately adjacent to the epithelial cell surface (Marteyn et al., 2010). Since FNR sites are also upstream of T3SS genes in *Yersinia* and *Salmonella* Enteritidis, FNR may play a similar role in these organisms.

A shift from aerobic to anaerobic conditions affects the cellular NADH/NAD^+ balance sensed by the repressor Rex in *S. aureus* and other Gram-positive bacteria. As NADH levels rise following the inhibition of aerobic respiration, Rex preferentially binds to NADH and no longer binds DNA, thus relieving repression of genes important for adaptation to anaerobiasis (Pagels et al., 2010). These include genes involved in lactate fermentation as well as nitrate and nitrite respiration (Ravcheev et al., 2012). A number of Rex-regulated genes are also part of the SrrA/SrrB regulon, thereby integrating input from multiple sensors.

Sensors for electron acceptors used for anaerobic respiration are also important for virulence during conditions of inflammation. The NarQ/NarP and NarX/NarL TCSs work together to control the expression of nitrate and nitrite reductases in response to nitrate and nitrite availability (Darwin and Stewart, 1995; Noriega et al., 2010). Nitrate typically forms during inflammation from the oxidation of NO· and serves as an important terminal electron acceptor in the absence of O_2. A *Salmonella narP* mutant exhibits impaired growth during colitis (Lopez et al., 2015). Methyl-accepting chemotaxis proteins are also important for survival in anaerobic host environments. For example, the chemoreceptor Trg senses glucose, ribose, and other carbohydrates, while the Tsr and Aer chemoreceptors mediate chemotaxis toward the electron acceptors nitrate and tetrathionate, respectively (Kondoh et al., 1979; Greer-Phillips et al., 2003; Rivera-Chávez et al., 2013). Tsr senses changes in PMF, and Aer monitors the redox state of the cell by means of an FAD-binding domain that undergoes a redox-sensitive conformational change. These responses are selectively important under conditions of inflammation and anaerobiasis, when the ability to respire on nitrate or tetrathionate is required (Rivera-Chávez et al., 2013).

Temperature

A shift from ambient environmental temperature to host body temperature can be an important signal for pathogens. Consequently, the expression of many virulence genes is thermoregulated. In Gram-negative bacteria, the histone-like protein H-NS and nucleoid-like protein Hha are involved in temperature-dependent regulation. In *Salmonella*, over 200 genes exhibit H-NS-dependent upregulation upon a shift from 25°C to 37°C, while in *E. coli*, nearly 70% of the 126 genes upregulated in response to temperature shift are also regulated by H-NS (Ono et al., 2005; White-Ziegler and Davis, 2009). At higher temperatures, the N terminus of H-NS undergoes a conformational change, leading to the formation of dimers rather than oligomers and a reduction in cooperative DNA binding (Ono et al., 2005). With a predilection for horizontally acquired DNA, H-NS regulates the expression of many virulence genes, including the temperature-responsive *Shigella* plasmid gene *virF* encoding a transcriptional activator of virulence genes, the *E. coli* hemolysin operon, and the *Salmonella* pathogenicity islands (Falconi et al., 1998; Madrid et al., 2002; Navarre et al., 2006). However, despite considerable evidence that temperature influences the expression of many H-NS-regulated genes, there are also many temperature-responsive genes that are not regulated by H-NS, as well as H-NS-regulated genes that are not differentially expressed in response to a change in temperature (Navarre et al., 2007). The relative AT content of genes is a better predictor of regulation by H-NS (Navarre et al., 2006; Lucchini et al., 2006).

Temperature is an important signal for many other pathogens, including *Listeria monocytogenes*, *Bordetella pertussis*, and *Yersinia pestis.* In *L. monocytogenes*, expression of flagellar genes is regulated by GmaR, a transcriptional anti-repressor whose stability is reduced at elevated temperatures (Kamp and Higgins, 2011); GmaR stability is enhanced through temperature-dependent interactions with the repressor MogR. Temperature sensing in *B. pertussis* is regulated through the BvgA/BvgS TCS. A shift to high temperature leads to an accumulation of the response regulator BvgA though an autoregulatory mechanism, which induces expression of several classes of virulence genes (Prugnola et al., 1995). At elevated temperatures, both H-NS and the counter-silencer RovA (a homolog of SlyA in *Salmonella*) have been implicated in the repression of *Y. pestis* gene expression. RovA relieves repression by H-NS but at high temperatures is rendered less able to bind DNA and more susceptible to proteolysis (Herbst et al., 2009; Quade et al., 2012). This mechanism is likely behind the temperature-dependent differences in

regulation by the PhoP/PhoQ and PmrA/PmrB TCSs in *Y. pestis* (Reinés et al., 2012).

In addition to regulatory proteins that respond to temperature through a change in conformation, RNA molecules can change conformation in response to temperature in order to regulate expression in a post-transcriptional manner (Narberhaus et al., 2006). Typically, the 5′ region of mRNA forms stable hairpin structures at lower temperatures that block ribosome access to the translational start site (Chowdhury et al., 2006). At elevated temperatures, these structures lose stability, allowing translation to proceed. Perhaps one of the best-characterized RNA sensors is the ROSE (repression of heat-shock gene expression) element. ROSE elements form three to four hairpin structures in the 5′ UTR of the mRNAs for small heat-shock proteins (Narberhaus et al., 2006). These elements have been principally characterized in the Rhizobiaceae, Gram-negative bacteria typically associated with soil and plants, although bioinformatic analysis has identified similar elements in α- and γ-proteobacteria, which include a wide array of bacteria, including some associated with plants and insects as well as mammalian pathogenic bacteria (Waldminghaus et al., 2005). The four U motif is a second type of RNA thermometer found in the 5′ UTRs of RNA heat-responsive genes in *Salmonella*. Four uracil residues base pair with the Shine-Dalgarno sequence to block ribosome access (Waldminghaus et al., 2007). A similar element is responsible for the observed temperature responsiveness of the transcription activator LcrF in *Y. pestis* and required for pathogenesis (Hoe and Goguen, 1993; Böhme et al., 2012). Additionally, a role for an RNA thermometer in virulence gene expression was previously described for the virulence regulator PrfA in *L. monocytogenes* (Johansson et al., 2002). The search for additional temperature-responsive RNA structures that regulate gene expression is ongoing (Righetti and Narberhaus, 2014).

Clinical Importance of Stress Responses

Many aspects of clinical host-pathogen interactions involve stress responses. Adaptive responses allow pathogenic bacteria to resist host defenses such as reactive oxygen and nitrogen species, antimicrobial peptides, and nutritional limitation during infection. Furthermore, cross-protection against environmental stress and antibiotics promotes bacterial resistance to therapeutic agents (Poole, 2012). As one example, activation of the AmgR/AmgS TCS of *P. aeruginosa* by membrane stress confers resistance to aminoglycosides that inhibit bacterial protein synthesis (Lee et al., 2009). Similarly, *Listeria*, *Bacillus*, *Staphylococcus*, *Salmonella*, and *Burkholderia* under nitrosative stress become highly resistant to aminoglycoside or β-lactam antibiotics (Vázquez-Torres and Bäumler, 2016). DNA damage resulting from oxidative or nitrosative stress leads to activation of the SOS stress response, which facilitates the development of antibiotic-tolerant persister cells (Wu et al., 2012) and can promote the horizontal dissemination of resistance genes (Beaber et al., 2004), as well as the expression of phage-encoded virulence factors (Kimmitt et al., 2000). The ability of certain antibiotics to elicit the SOS response is believed to account for the development of the hemolytic-uremic syndrome in some patients following antibiotic treatment of EHEC infections (Wong et al., 2000).

Stress responses also play a critically important role in the evolution of pathogenic bacteria. The regulatory protein OmpR is responsive to acid pH in *Salmonella*, but not in *E. coli* (Quinn et al., 2014), which has led to substantial divergence in the composition of the OmpR regulons in these related bacterial species and a specialized role for *Salmonella* OmpR in the intraphagosomal environment (Chakraborty et al., 2015). Longitudinal analysis of *B. pseudomallei* and *P. aeruginosa* during chronic pulmonary infections has demonstrated numerous mutations involving stress-response regulators (Damkiær et al., 2013; Price et al., 2013). For instance, the mucoid phenotype characteristic of *P. aeruginosa* isolates from patients with cystic fibrosis results from mutations involving the σ^E stress pathway (Boucher et al., 1997), which allow the bacteria to persist despite the presence of a chronic inflammatory response. In pathogenic streptococci, the CovR/CovS TCS responds to a variety of environmental stresses including iron deprivation and antimicrobial peptides (Froehlich et al., 2009). CovR/CovS negatively regulates a number of important virulence factors, and *covRS* mutants exhibit enhanced cytotoxicity and invasiveness. The development of invasive streptococcal infections is frequently associated with the emergence of *covRS* mutants, suggesting that serious illness caused by these organisms arises from regulatory mutations that disrupt a stable commensal host-bacterial interaction (Kreikemeyer et al., 2003; Patras et al., 2013).

Concluding Remarks

Pathogenic bacteria are able to sense and respond to diverse microenvironmental stresses encountered during infection. These responses allow pathogens not only to withstand specific stressful conditions but also to express virulence-associated genes in a spatiotemporally appropriate manner. A detailed understanding of bacterial stress responses provides novel insights into the nature of host microenvironments, mechanisms of virulence and stress resistance, and potential targets for intervention for the prevention or treatment of infectious diseases.

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