Chapter 7
Molecular Profiling: Catecholamine Modulation of Gene Expression in *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium

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Abstract Investigations of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium have demonstrated that these bacterial pathogens can respond to the presence of catecholamines including norepinephrine and/or epinephrine in their environment by modulating gene expression and exhibiting various phenotypes. For example, one of the most intensively investigated phenotypes following exposure of *E. coli* and *S.* Typhimurium to norepinephrine is enhanced bacterial growth in a serum-based medium. Host-pathogen investigations have demonstrated that the mammalian host utilizes nutritional immunity to sequester iron and prevent extraintestinal growth by bacterial pathogens. However, *Salmonella* and certain *E. coli* strains have a genetic arsenal designed for subversion and subterfuge of the host. Norepinephrine enhances bacterial growth due, in part, to increased iron availability, and transcriptional profiling indicates differential expression of genes encoding iron acquisition and transport proteins. Bacterial motility of *E. coli* and *S.* Typhimurium is also enhanced in the presence of catecholamines and increased flagellar gene expression has been described. Furthermore, epinephrine and norepinephrine are chemoattractants for *E. coli* O157:H7. In *S.* Typhimurium, norepinephrine enhances horizontal gene transfer and increases expression of genes involved in plasmid transfer. Exposure of *E. coli* O157:H7 to norepinephrine increases expression of the genes encoding Shiga toxin and operons within the locus of enterocyte effacement (LEE). Alterations in the transcriptional response of enteric bacteria to catecholamine exposure in vivo are predicted to enhance bacterial colonization and pathogen virulence. This chapter will review the current literature on the transcriptional response of *E. coli* and *S.* Typhimurium to catecholamines.

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7.1 Introduction

The ability of bacteria to respond to the catecholamines epinephrine (Epi) and norepinephrine (NE) has stimulated intense interest in the field of microbial endocrinology for greater than a decade. In the family *Enterobacteriaceae*, *Escherichia coli* and *Salmonella enterica* have been investigated most frequently. Multiple phenotypes have been described for both *E. coli* and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) in response to Epi and NE. The utilization of microbial endocrinology by *Salmonella* and certain *E. coli* strains to acquire iron in vivo and subvert nutritional immunity imposed by the mammalian host is perhaps the phenotype that is best understood. However, additional phenotypes including Epi-/NE-enhanced motility, virulence gene regulation, and horizontal gene transfer are equally intriguing. This chapter will focus on the current knowledge of transcriptional profiling in *Escherichia coli* O157:H7 (O157:H7) and *S.* Typhimurium.

7.2 *Salmonella enterica*

7.2.1 Experimental Conditions

Multiple laboratories have investigated the response of *Salmonella enterica* serovar Typhimurium to the catecholamines epinephrine or norepinephrine. These investigations often utilized different strain backgrounds, media, and different concentrations of catecholamines. For example, the concentration of NE in different investigations ranges from 5 μM to 5 mM. Therefore, a direct comparison between individual experiments is difficult and may not be scientifically appropriate. Pullinger et al. (Pullinger et al. 2010a) utilized serum-SAPI medium with 5 mM NE and Dulbecco's modified Eagle's medium (DMEM) containing either 5 or 50 μM NE. Investigations by Bearson and colleagues utilized serum-SAPI medium containing 50 μM or 2 mM NE and DMEM motility medium containing 50 μM or 100 μM NE (Bearson and Bearson 2008; Bearson et al. 2008; Bearson et al. 2010). Karavolos et al. utilized LB medium containing 50 μM adrenaline (epinephrine, EPI) (Karavolos et al. 2008). Merighi et al. utilized DMEM motility medium containing 50 μM Epi (Merighi et al. 2009). The Sperandio laboratory has utilized LB medium containing 50 μM NE (Moreira et al. 2010; Rasko et al. 2008) and LB and N-minimal medium containing 50 μM Epi (Moreira and Sperandio 2012).

7.2.2 The QseBC Two-Component System

The regulatory system most frequently investigated in catecholamine-enhanced phenotypes is the QseBC two-component system with QseC as the sensor kinase and QseB serving as the response regulator. A point of contention in this subject area concerns the role of the QseBC two-component system in NE and Epi signaling; specifically at least four laboratories have investigated the response of *S.* Typhimurium motility to catecholamines without a clear consensus concerning whether QseC is involved. For example, Bearson et al. indicated that wild-type *S.* Typhimurium, as well as *qseC*, *qseB*, and *qseBC* mutants have a significant increase in motility on DMEM in response to 50 μM NE (Bearson et al. 2010). However, although motility of a *qseC* mutant significantly increases in response to NE exposure, the motility of a *qseC* mutant is significantly decreased compared to wild-type in the presence or absence of 50 μM NE. The bacterial motility of *qseB* and *qseBC* mutants are either similar or slightly greater than wild-type in both the presence or absence of 50 μM NE. Therefore, data from Bearson et al. indicates that the QseB response regulator is a negative repressor of bacterial motility with the motility repression phenotype of the QseBC two-component system dependent upon the presence of QseB in the absence of *qseC*, but NE-enhanced motility of *S.* Typhimurium did not require QseBC (Bearson et al. 2010). Moreira et al. demonstrated that *S.* Typhimurium motility was significantly enhanced on LB medium in the presence of 50 μM NE; the Authors indicate that NE enhanced the bacterial motility of a *qseC* mutant, but this difference was not statistically significant compared to the absence of NE (Moreira et al. 2010). Merighi et al. demonstrated that, although 50 μM Epi enhanced the motility of both wild-type *S.* Typhimurium and a *qseC* (*preB*) mutant on DMEM, this difference was not statistically significant (Merighi et al. 2009). However, this study did indicate that Epi-enhanced motility was statistically significant for both *qseB* (*preA*) and *qseBC* (*preAB*) mutants. Due to the Epi-enhanced motility of the *qseBC* mutant on DMEM, Merighi et al. indicated that the Epi response was not mediated by QseB/QseC. An investigation by Pullinger et al. indicated that in contrast to some of the above mentioned studies, neither wild-type *S.* Typhimurium nor a *qseC* mutant consistently responded to NE on DMEM motility medium (Pullinger et al. 2010a). Furthermore, the *qseC* mutant investigated by Pullinger et al. did not have decreased motility compared to wild-type *S.* Typhimurium, another contrast from other publications. One potential reason for discrepancies concerning the role of catecholamines in *S.* Typhimurium motility could be the ability of Epi and NE to bind iron with different media and their sources having various concentrations of iron. NE can function as a siderophore, and Bearson et al. demonstrated that *S.* Typhimurium motility was enhanced on DMEM with exogenous addition of iron (Freestone et al. 2000; Bearson et al. 2010); maximal bacterial motility was achieved by *S.* Typhimurium in the presence of 100 μM NE and 80 μM $FeCl_3$. Due to the contentious role of *S.* Typhimurium QseC sensor kinase in catecholamine signaling, the description of gene expression in response to Epi and NE will be predominately devoted to wild-type *S.* Typhimurium.

7.2.3 Norepinephrine-Enhanced Motility

Norepinephrine-enhanced motility of *S.* Typhimurium has been demonstrated on DMEM medium containing 0.3 % agar (Bearson and Bearson 2008). Furthermore, transcriptional analysis of *S.* Typhimurium in the presence of 2 mM NE (Bearson and Bearson 2008) in serum-SAPI minimal medium using DNA microarrays and real-time RT-PCR analyses indicated that a number of flagellar and chemotaxis genes were up-regulated during NE exposure. Flagellar assembly is a complex process and requires the coordination of a cascade of early, middle and late genes for the production of gene products that ultimately result in flagellar assembly for motility (Chilcott and Hughes 2000). This hierarchy results in the amplification of the signal such that the relative level of expression is "late">"middle">"early" genes. For example, in the presence of 2 mM NE, the *S.* Typhimurium genes *fljB* (late), *fliY* (middle), *fliA* (middle) and *flhC* (early) are significantly induced 15.4-, 4.2-, 3.6-, and 1.4-fold compared to the absence of NE, respectively (Bearson and Bearson 2008). Moreira et al. also demonstrated that the transcription of *flhDC* encoding the master regulators of motility was significantly increased in LB medium in the presence of 50 μM NE compared to the absence of NE (Moreira et al. 2010). Microarray analysis by Karavolos demonstrated that *flgD* (encoding flagellar hook capping protein) was down-regulated in the presence of Epi (Karavolos et al. 2008).

7.2.4 Nutritional Immunity, Norepinephrine-Enhanced Growth, and Iron Utilization

Differential expression of iron-regulated genes of *S.* Typhimurium was demonstrated in LB medium by Karavolos et al. using microarray analysis (Karavolos et al. 2008). Specifically, *fhuAC*, *exbBD*, *entE*, *feoAB*, and *sitAB* were up-regulated and *ftn* (ferritin) was down-regulated in the presence of 50 μM Epi.

Norepinephrine-enhanced growth of *S.* Typhimurium in serum-SAPI minimal medium is due to the siderophore-like activity of NE in the presence of transferrin (Freestone et al. 1999). Bacteria require optimal concentrations of iron for growth and either excessive or insufficient quantities of iron can be toxic or inhibitory to bacteria, respectively. The ability of serum derived transferrin to sequester iron from the bacterial cell creates an iron-deplete environment resulting in no or slow growth depending on the concentration of bacterial cells. Transcriptional analysis using microarrays to monitor gene expression of *S.* Typhimurium grown in serum-SAPI medium containing 2 mM NE compared to the absence of NE confirms the iron-deplete environment of serum-SAPI medium since transcription of genes encoding iron uptake and utilization pathways are decreased in the presence of NE (Bearson et al. 2008). This indicates that NE scavenges iron from the environment in a siderophore-like manner and increases iron availability to the bacterial cell, resulting in NE-enhanced growth of *S.* Typhimurium and a decreased need for the

production of iron uptake and utilization proteins. Interestingly, although the expression of genes encoding iron acquisition proteins is down-regulated in the presence of NE, a subset of these iron uptake proteins are required for NE-enhanced growth of *S.* Typhimurium (Williams et al. 2006; Bearson et al. 2008). This suggests that the relative expression of iron acquisition genes is lower in the presence of NE but these genes are not in a transcriptional "off" state. In addition, the microarray experiments were performed during the exponential phase ($O.D._{600}=0.4$) of bacterial growth when enterochelin, salmochelin and their breakdown products are accumulating in the growth medium. Since enterochelin/salmochelin production is necessary for NE-enhanced growth of *S.* Typhimurium (Bearson et al. 2008), the accumulation of these siderophores in the presence of NE increases iron availability which concomitantly reduces the expression of genes encoding iron acquisition proteins via the iron regulator Fur.

Norepinephrine-enhanced growth in serum-SAPI minimal medium is a model of in vivo growth during systemic infection of the mammalian host. Nutritional immunity is used by the host to limit iron availability and suppress bacterial pathogen growth (Hood and Skaar 2012). As previously described, transferrin present in serum sequesters iron from the bacterial cell and prevents bacterial growth (Fig. 7.1). However, NE can assist the bacterial cell by providing iron in the presence of enterochelin/salmochelin (Burton et al. 2002; Freestone et al. 2000; Freestone et al. 2003). A countermeasure is deployed by the host immune system using siderocalin (lipocalin 2) to sequester enterochelin (Goetz et al. 2002). *S.* Typhimurium's defense is the synthesis of salmochelin via glucosylation of enterochelin which prevents binding to siderocalin (Fischbach et al. 2006; Smith 2007; Hantke et al. 2003). In addition to the binding by siderocalin, enterochelin has a high membrane affinity which results in membrane sequestration (Luo et al. 2006). Glucosylation of enterochelin to salmochelin by IroB and hydrolysis of salmochelin by the periplasmic hydrolase IroE decreases membrane affinity and increases the iron acquisition rate for *S.* Typhimurium. Therefore the ability of *S.* Typhimurium to produce, transport and breakdown salmochelin via products of the *iroA* gene cluster is a virulence hallmark that assists *S.* Typhimurium in causing systemic disease (Fischbach et al. 2005; Smith 2007; Luo et al. 2006). This is in contrast to most *E. coli* strains, except a subset that also contain the *iroA* gene cluster including uropathogenic *E. coli* (Hantke et al. 2003).

The ability of NE to enhance the growth of *S.* Typhimurium in serum-SAPI minimal medium (Freestone et al. 1999; Williams et al. 2006; Bearson et al. 2008) suggests that additional biosynthetic pathways would be modulated besides the iron utilization and transport genes. Transcriptional analysis using microarrays on *S.* Typhimurium grown in serum-SAPI medium containing 2 mM NE revealed that NE exposure increases transcription of genes involved in amino acid biosynthesis, cofactor biosynthesis, central intermediary metabolism, energy metabolism, and synthesis of transport and binding proteins (Bearson et al. 2008). Thus, to take advantage of the increased availability of iron provided by NE in serum-SAPI minimal medium, *S.* Typhimurium modulates the biosynthesis of multiple cellular pathways to increase growth rate.

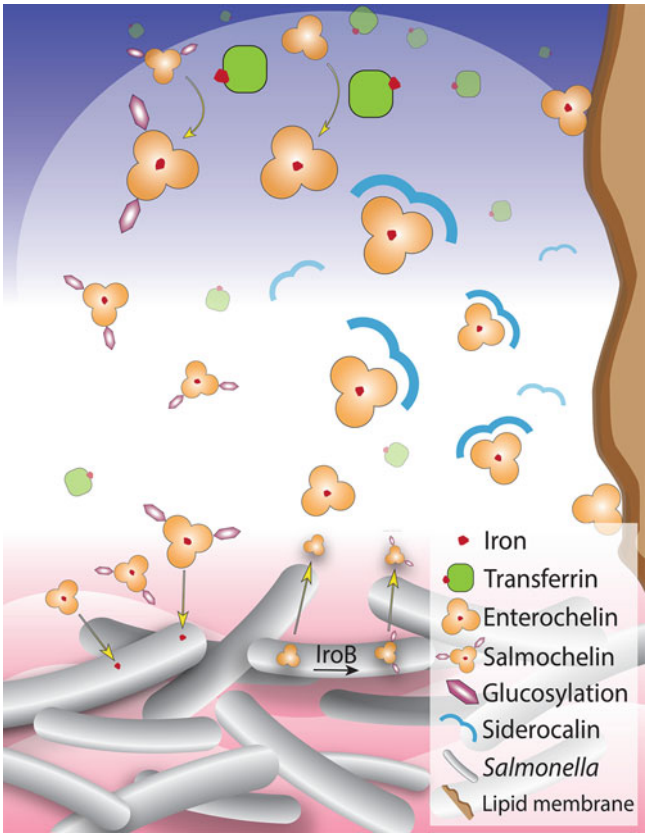

Fig. 7.1 Nutritional Immunity: a competition between mammalian iron sequestration and bacterial iron acquisition that influences bacterial growth proficiency in the mammalian host. Transferrin and other host iron binding proteins sequester iron, thereby preventing growth of microorganisms at systemic locations. Members of the *Enterobacteriaceae* family including *Salmonella enterica* produce enterochelin, a siderophore with a high affinity for iron. The bioavailability of enterochelin for bacterial iron acquisition is reduced by the mammalian siderocalin as well as the affinity of enterochelin for lipid membranes. *Salmonella* glucosylation of enterochelin to salmochelin by IroB reduces both membrane affinity and siderocalin binding. The increased bioavailability of salmochelin compared to enterochelin facilitates iron acquisition from transferrin to support pathogen growth at systemic sites. Epinephrine/norepinephrine are not required for bacterial iron acquisition in vivo. However, due to their siderophore-like properties, Epi/NE accelerate bacterial iron acquisition and therefore when present can enhance pathogen growth in iron-limited environments. (Illustration by Michael Marti)

7.2.5 SPI-1 and Invasion

Genes encoded in *Salmonella* pathogenicity island 1 (SPI-1) are important for invasion of eukaryotic cells. Moreira and Sperandio demonstrated a 1.5-fold increase in the invasion of HeLa cells by wild-type *S.* Typhimurium in the presence of 50 μM

Epi compared to the absence of the catecholamine (Moreira and Sperandio 2012). For quantitative real-time RT-PCR, cultures were grown aerobically to late log phase ($O.D._{600} = 1.0$) in LB medium with or without 50 μM NE or Epi (Moreira et al. 2010; Moreira and Sperandio 2012). Transcription of *invF* and *sopB* encoded in SPI-1 were increased >15-fold in LB medium in the presence of 50 μM NE compared to the absence of NE, whereas 50 μM Epi increased *sopB* and *sipA* transcription ~twofold in LB compared to the absence of Epi. In contrast to Moreira and Sperandio, the microarray analysis by Karavolos et al. following 30 min of exposure to 50 μM Epi (adrenaline) in LB medium during late log phase ($O.D._{600} \sim 1.0$) indicated that *invF* (encoding an invasion protein) was down-regulated (Karavolos et al. 2008). Pullinger et al. using a *prgH-gfp* reporter strain in serum-SAPI medium with and without 5 mM NE indicated that the transcription of *prgH* was low and not differentially expressed (Pullinger et al. 2010a).

7.2.6 Salmonella *Pathogenicity Island 2 (SPI-2) and SPI-2 Effectors*

Intracellular survival of *Salmonella* in epithelial cells and macrophages requires genes encoded within *Salmonella* pathogenicity island 2 (SPI-2) and additional SPI-2 effectors located outside of SPI-2 (Figueira and Holden 2012). Transcription of *ssaG* from a *gfp* transcriptional fusion was significantly reduced in *S.* Typhimurium cultures grown 16 h in serum-SAPI medium containing 5 mM NE compared to medium without NE (Pullinger et al. 2010a). The authors also demonstrated a reduction in *ssaG* expression in the presence of 25 μM Fe (III) and noted that this reduction was similar to the effect of NE. Growth of wild-type *S.* Typhimurium in LB medium to late log in the presence of 50 μM NE enhanced transcription of *sifA* greater than fivefold compared to LB medium in the absence of NE (Moreira et al. 2010; Rasko et al. 2008). A similar threefold induction of transcription was seen for cultures grown to late log in N-minimal medium pH 4.5 in response to 50 μM Epi exposure (Moreira and Sperandio 2012).

7.2.7 *PmrAB Regulon*

Members of the PmrAB regulon (*pmrFGHIJ*) were down-regulated in the presence of Epi based on microarray analysis (Karavolos et al. 2008). The PmrAB two-component signal transduction system is important for *Salmonella* resistance to antimicrobial peptides and virulence (Gunn 2008). Extracellular iron has been shown to activate the PmrAB regulon for prevention of iron toxicity (Wosten et al. 2000). The decreased expression of the PmrAB regulon may be due to sequestration of extracellular iron by Epi which would decrease iron binding and signaling by the PmrB sensor kinase.

7.2.8 *Epi- and NE-Regulated Genes Identified by Transposon Mutagenesis*

A screen of 10,000 *S.* Typhimurium Mu*dJ* transposon mutants identified seven fusions down-regulated and one fusion up-regulated in the presence of ~250 μM Epi (Spencer et al. 2010). The down-regulated genes included *virK*, *mig14*, *iroC* (see NE-enhanced growth above), *accC*, *nrdF*, *yedP*, and STM3081; the *yhaK* gene was up-regulated. Analysis of transcriptional activity using β-galactosidase assays following a 30 min exposure during mid-log phase to either 50 μM Epi or NE was confirmed in M9 minimal medium for down-regulated genes and LB medium for the up-regulated gene, *yhaK*. The regulation of *yhaK*, *virK*, and *mig14* by 500 μM Epi and NE could be reversed in a promoter-luciferase fusion assay by addition of 500 μM phentolamine, an α-adrenergic antagonist. Both *virK* and *mig14* are *Salmonella* virulence genes involved in bacterial resistance to antimicrobial peptides (Brodsky et al. 2002; Detweiler et al. 2003). Exposure to 500 μM Epi or NE significantly increased sensitivity of wild-type *S.* Typhimurium to the antimicrobial peptide cathelicidin LL-37 (Spencer et al. 2010). Furthermore, a significant increase in sensitivity to LL-37 was demonstrated for the *virK* mutant compared to wild-type *S.* Typhimurium in the absence of catecholamines.

7.2.9 *Horizontal Gene Transfer*

Exposure to 5 μM NE in LB medium significantly increased conjugation frequency of a plasmid encoding multidrug resistance from a donor *S.* Typhimurium strain to a recipient *E. coli* strain (Peterson et al. 2011). Enhanced plasmid transfer was associated with a significant up-regulation of *tra* gene expression involved with plasmid transfer; specifically, the transcription of *traGIJRY* was increased upon exposure to NE. Treatment with 500 μM phentolamine reduced the NE-enhanced conjugation frequency to baseline levels as did propranolol but this effect was delayed. Treatment with adrenergic antagonists did not reduce the baseline conjugation frequency of the *S.* Typhimurium donor. This study suggests that bacterial exposure to catecholamines may influence the evolution and adaptation of pathogens in the environment due to the transfer of genes that encode resistance to antibiotics and virulence factors.

7.3 *Escherichia coli*

7.3.1 *Experimental Conditions*

Investigations of *E. coli* exposure to catecholamines have also used various media and concentrations of catecholamines. The experimental conditions for DNA microarray analysis by Dowd involved a 1:50 dilution of an *E. coli* O157:H7 EDL933

overnight culture in serum-SAPI medium with or without 50 μM NE (Dowd 2007). The EDL933 culture was harvested following incubation for 5 h at 37 °C, 0.05 % CO_2, 95 % humidity. Bansal et al. also utilized EDL933 to analyze biofilm gene expression using microarrays in the presence of 50 μM Epi, NE, or untreated controls (Bansal et al. 2007). The EDL933 biofilms were developed for 7 h on 10 g of glass wool in 250 ml LB, 0.2 % glucose with a starting bacterial turbidity of ~0.03. DNA microarrays were employed to transcriptionally analyze *E. coli* O157:H7 86-24 harvested for RNA extraction at $O.D._{600} = 1.0$ following growth in low-glucose Dulbecco's modified Eagle's medium (DMEM) with and without 50 μM Epi at 37 °C with shaking at 250 rpm (Njoroge and Sperandio 2012).

7.3.2 Motility

Bacterial motility in the presence of Epi or NE is one of the most often investigated phenotypes of *E. coli* in response to catecholamines. Using an agarose plug chemotaxis assay, both Epi and NE were chemoattractants in a concentration-dependent migration of EDL933 towards the catecholamines (Bansal et al. 2007). Furthermore, Epi and NE increased motility 1.4-fold compared to the control culture on motility medium containing 1 % tryptone and 0.25 % NaCl. Microarray analysis of EDL933 biofilm cultures in the presence of Epi and NE by Bansal et al. demonstrated a significant increase in *fliD* encoding a flagellar hook-associated protein but a decrease in *motB* encoding a subunit for the flagellar proton motive force generator. Transcription of *fliC* encoding a flagellin protein was significantly increased approximately twofold in the presence of 50 μM Epi in DMEM compared to cultures without catecholamines (Rasko et al. 2008). The presence of 50 μM NE has been shown in multiple investigations to significantly enhance the motility of *E. coli* O157:H7 86-24 in DMEM motility assays (Sharma and Casey 2014a, b). However, an increase in the transcription of genes in the flagellar and chemotaxis operons is not always apparent, probably due to the experimental conditions used. Gene expression assays are usually performed using broth cultures whereas motility assays are typically performed using semi-solid agar medium. The differences in incubation conditions including growth rate and growth phase for broth and motility assays may account for a lack of congruence between transcriptional analysis and motility phenotype.

7.3.3 The QseBC Two-Component System

The role of regulatory proteins in catecholamine enhanced phenotypes including motility is an area of intense research. The QseC sensor kinase has been proposed to be a bacterial adrenergic receptor (Clarke et al. 2006). Multiple investigations using EHEC and UPEC isolates have demonstrated that *qseC* mutants have decreased motility compared to wild-type *E. coli* (Sperandio et al. 2003; Hughes et al. 2009;

Kostakioti et al. 2009; Hadjifrangiskou et al. 2011; Guckes et al. 2013). Inactivation of the *qseC* gene in the presence of an active QseB response regulator results in pleiotropic effects including virulence attenuation, metabolic dysregulation and decreased motility (Kostakioti et al. 2009; Hadjifrangiskou et al. 2011). Multiple physiological pathways are perturbed in *qseC* mutants, including a compromised TCA cycle. Interrogation of the TCA cycle in *E. coli* UPEC revealed that Δ*sdhB* and Δ*mdh* mutations confer virulence attenuation and decreased motility compared to the wild-type strain (Hadjifrangiskou et al. 2011); these phenotypes are similar to those of a *qseC* mutant, indicating that the presence of an active QseB in the absence of QseC results in decreased motility and metabolic dysregulation. In support of this hypothesis, *qseB* mutants of both EHEC and UPEC do not have decreased motility or virulence attenuation compared to wild-type *E. coli* (Hughes et al. 2009; Kostakioti et al. 2009). Furthermore, Sharma and Casey demonstrated that an *E. coli* O157:H7 *qseBC* mutant has a similar motility phenotype on DMEM motility medium compared to wild-type O157:H7 (Sharma and Casey 2014b). However, in response to 50 μM NE, Sharma and Casey demonstrated a significant increase in the motility of *E. coli* O157:H7 *qseC* and *qseBC* mutants (Sharma and Casey 2014a, b). These results suggest that either QseC is not a sensor for catecholamines or as has been suggested, multiple regulatory systems sense and response to Epi and NE in *E. coli* (Njoroge and Sperandio 2012; Karavolos et al. 2013). Due to the pleiotropic effects displayed by a *qseC* mutant, a clear consensus concerning the role of specific two-component systems in catecholamine sensing is lacking. For this reason, differential expression of genes regulated by the QseBC and other two-component systems in response to catecholamines will not be further discussed for *E. coli*. Instead, readers are referred to publications by Hughes et al. and Njoroge and Sperandio for further information (Hughes et al. 2009; Njoroge and Sperandio 2012).

7.3.4 E. coli *O157:H7 Locus of Enterocyte Effacement (LEE)*

The locus of enterocyte effacement (LEE) is a pathogenicity island that contains multiple operons and is present in enteropathogenic (EPEC) and various enterohemorrhagic *E. coli* (EHEC). As shown by quantitative RT-PCR, exposure of EHEC strain 86-24 to 50 μM Epi increased the expression of the *ler* gene encoding a regulator of LEE expression by ~1.5-fold (Rasko et al. 2008). Microarray analysis and quantitative RT-PCR by Dowd demonstrated that the most highly induced genes in EDL933 due to 50 μM NE exposure were *espAB* encoded in the LEE4 operon (Dowd 2007). The *eae* gene in the TIR operon was also highly expressed in the presence of NE. Using microarrays Njoroge and Sperandio indicated that exposure of 86-24 to 50 μM Epi increased the expression of LEE genes and non-LEE encoded virulence effectors (Njoroge and Sperandio 2012). Quantitative RT-PCR confirmed that expression of the LEE effector *espA* and the non-LEE effector *nleA* were increased two- and sixfold in the presence of Epi, respectively.

7.3.5 Shiga Toxin and the SOS Response

The shiga toxins Stx1 and Stx2 are encoded within lambdoid prophages integrated into the chromosome of *E. coli* O157:H7 strains. Similar to other phage-encoded genes, the regulation of *stx1* and *stx2* may be stimulated by environmental stresses that induce an SOS response. Microarray analysis by Dowd in serum-SAPI medium demonstrated that transcription of *stx1*, *stx2, umuD*, *recB*, and several phage-encoded gene products (endolysins and holin) were increased in EDL933 due to 50 μM NE. Induction of the SOS response is typically due to environmental stresses that induce DNA damage and the concomitant response to repair damage to nucleic acids. One possible explanation for induction of DNA damage is the enhanced bioavailability of iron due to NE with elevated intracellular iron increasing the vulnerability of bacterial DNA to oxidative damage (Touati et al. 1995). Dowd also suggested that NE could be an inducer of a positive adaptive state.

7.3.6 Iron Acquisition

Regulation of iron uptake and utilization genes is a common theme following exposure to catecholamines, and microarray analysis by both Dowd and Bansal et al. confirmed this effect (Dowd 2007; Bansal et al. 2007). Exposure of biofilms by Bansal et al. to 50 μM Epi or NE induced *feoAB*, *fhuBCD*, and additional iron regulated genes. Cultures of EDL933 grown in serum-SAPI medium in the presence of 50 μM NE increased expression of *fecCD*, *fhuD*, and *feoB*; other iron-regulated genes were down-regulated by NE including *fepAC*, *entCD*, and the ferric uptake regulator *fur*. The results of Dowd are consistent with the iron deplete conditions of serum-SAPI medium due to transferrin present in mammalian serum that sequesters iron from the bacterial cell in opposition to the property of NE to bind iron and promote the growth of *E. coli*.

7.3.7 Cold Shock

Investigations by both Bansal et al. and Dowd found that genes encoding cold shock proteins were induced due to exposure to 50 μM Epi and NE (Bansal et al. 2007; Dowd 2007). Bansal et al. demonstrated that the expression of the cold shock regulator genes *cspGH* were increased 6- to 23-fold due to the presence of Epi or NE. In addition, the cold shock genes *cspE* and *deaD* were also up-regulated due to catecholamine exposure. Dowd demonstrated that the expression of *cspG* and *cspH* increased 3.4- and 3.6-fold in the presence of NE, respectively.

7.4 Prospective Research

Investigation of microbial endocrinology is still in its infancy with fundamental research having demonstrated that microorganisms, including certain pathogens, can sense and respond to mammalian hormones. Most of the research investigating microbial endocrinology has been performed in vitro utilizing single hormones and analysis of individual bacterial phenotypes as a surrogate for complex environments found in nature and the mammalian host. Therefore, our current understanding of the bacterial phenotypes associated with microbial endocrinology tend to be one dimensional with a lack of knowledge concerning phenotypic networks that are expressed in response to a hormone signal. For the few investigations that have analyzed global gene expression patterns, our understanding of the interaction between bacterial regulons and stimulons is obscured by a list of genes with unknown functions. A future challenge is to understand the response of enteric bacteria to multiple, concurrent signals including hormones and environmental signals that may influence gene expression cooperatively or antagonistically. This will require additional knowledge concerning signals in a given environmental niche and the elucidation of roles for differentially expressed genes of unknown function. An example is provided below of the requirement for additional information in order to enhance our understanding and integrate multiple signals in a complex environment.

Swine are often asymptomatically colonized with *Salmonella,* and it has been known for greater than 40 years that stress (transportation, feed withdrawal, social, etc.) increases *Salmonella* fecal shedding in pigs colonized with the pathogen (Williams and Newell 1970). Recently, two investigations have demonstrated that mammalian stress hormones are involved in *Salmonella* recrudescence and can increase fecal shedding in swine. Pullinger et al. demonstrated that 6-hydroxydopamine (6-OHDA) administration to swine increased fecal shedding of pigs colonized with *Salmonella* in a porcine model of colonization (Pullinger et al. 2010b). Administration of 6-OHD, a selective neurotoxin, releases NE into the gastrointestinal tract by destroying noradrenergic nerve terminals. Increased *Salmonella* shedding in swine following 6-OHD administration suggests that norepinephrine release during animal stress increases *Salmonella* recrudescence/shedding in animals already colonized with the pathogen. Using a feed withdrawal stress model for *Salmonella*-colonized swine, Verbrugghe et al. demonstrated that stress increases serum cortisol levels with an associated recrudescence in *Salmonella* shedding (Verbrugghe et al. 2011). Also, exposure of alveolar macrophages in vitro to cortisol increased *Salmonella* intracellular proliferation. The increased bacterial proliferation did not occur following exposure of alveolar macrophages to NE or dopamine. In addition, the increased *Salmonella* proliferation in alveolar macrophages in response to cortisol was not associated with direct exposure of *Salmonella* to the hormone, and microarray analysis indicated that bacterial genes were not differentially expressed in response to cortisol exposure. The studies by Pullinger et al. and Verbrugghe et al. indicate that multiple mammalian hormones, including Epi, NE, and cortisol, may stimulate *Salmonella* recrudescence in swine. These and potentially other hormones or host

factors (such as cytokines) may have both direct and indirect effects on host cells, pathogens, and the host microbiota to influence the magnitude and duration of pathogen colonization and shedding. To date, investigations of microbial endocrinology have focused on an individual hormone (such as NE or cortisol) and an individual phenotype (such as motility or recrudescence). An integrated understanding of the host/bacterium relationship in the context of the complex host has yet to be realized. Tissue specific investigations of the host/pathogen response to microbial endocrinology are currently possible (for example, utilizing RNAscope), but a lack of knowledge concerning the most appropriate host tissues and potential genetic pathways to target for analysis limit research progress. With technological advances in nucleotide sequencing, imaging, proteomics, and metabolomics, future investigations should be capable of interrogating multiple interactions that influence both eukaryotic and prokaryotic cells that culminate in phenotypes that have already been described, as well as others yet to be discovered.

7.5 Concluding Remarks

Research investigations into the bacterial phenotypes induced by catecholamine exposure of *E. coli* and *Salmonella enterica* continue to emerge in conjunction with the associated gene expression profiles. To date the phenotype that is best understood is increased bioavailability of iron due to Epi-/NE-enhanced bacterial growth in serum containing medium. Multiple catecholamine sensor/regulators have been proposed by various investigators but our current knowledge concerning their relative importance is partially obscured due to pleiotropic effects. Continued research should clarify the role of catecholamine sensor/regulators in gene modulation by minimizing non-specific consequences.

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