Microbiology Spectrum
American Society for Microbiology Press

# Enterohemorrhagic *Escherichia coli* Virulence Gene Regulation

JAY L. MELLIES[1] and EMILY LORENZEN[1]
[1]Department of Biology, Reed College, Portland, OR 97202

Q1

**ABSTRACT** Coordinated expression of enterohemorrhagic *Escherichia coli* virulence genes enables the bacterium to cause hemorrhagic colitis and the complication known as hemolytic-uremic syndrome. Horizontally acquired genes and those common to *E. coli* contribute to the disease process, and increased virulence gene expression is correlated with more severe disease in humans. Researchers have gained considerable knowledge about how the type III secretion system, secreted effectors, adhesin molecules, and the Shiga toxins are regulated by environmental signals and multiple genetic pathways. Also emergent from the data is an understanding of how enterohemorrhagic *E. coli* regulates response to acid stress, the role of flagellar motility, and how passage through the human host and bovine intestinal tract causes disease and supports carriage in the cattle reservoir, respectively. Particularly exciting areas of discovery include data suggesting how expression of the myriad effectors is coordinately regulated with their cognate type III secretion system and how virulence is correlated with bacterial metabolism and gut physiology.

As a species, *Escherichia coli* is highly successful, adapting to inhabit the lower intestine of warm-blooded animals. Commensal *E. coli*, part of the normal biota, resides harmlessly in the gut, producing vitamin K. However, *E. coli* also causes three types of disease in humans: urinary tract infections, sepsis in newborns, and diarrheal disease. Enterohemorrhagic *E. coli* (EHEC) plays a prominent role in the third type of illness. It has been estimated that, for the pan genome of *E. coli*, the nonpathogenic and pathogenic strains only contain a core set of genes comprising approximately 20% of any one genome (1, 2). Much of the horizontally acquired genetic information is clustered within genomic islands in pathogens. As for EHEC, this has allowed the organism to not only attach and colonize the large intestine of humans and other animals, to outcompete commensal *E. coli* and other bacteria at the site of infection, but also to cause serious disease.

Horizontally acquired genetic information in EHEC results in evolution into a specific pathotype—genotype dictates phenotype. How this genetic information is controlled is of equal importance for the success and virulence of the organism. Indeed, Abu-Ali et al. (3) investigated differences in virulence gene regulation in two distinct EHEC isolate lineages, clade 8 and clade 2. A clade is a group of EHEC isolates with one ancestor and all its descendants. Eight clades of *E. coli* O157 isolates were defined by single nucleotide polymorphism (SNP) analyses, where clade 8 was a group of hypervirulent bacteria compared to the other seven clades (4). By examining multiple strains per lineage, the investigators found increased expression of horizontally acquired virulence genes in clade 8 versus clade 2. Genes expressed to higher levels in clade 8, which is associated with a greater number of cases of *E. coli* hemorrhagic

**Received:** 30 April 2013, **Accepted:** 21 August 2013,
**Published:** ••••••
**Editors:** Vanessa Sperandio, University of Texas Southwestern Medical Center, Dallas, TX, and Carolyn J. Hovde, University of Idaho, Moscow, ID
**Citation:** Mellies JL, Lorenzen E. 2014. Enterohemorrhagic *Escherichia coli* virulence gene regulation. *Microbiol Spectrum* 2(4): EHEC-0004-2013. doi:10.1128/microbiolspec.EHEC-0004-2013.
**Correspondence:** Jay L. Mellies, jay.mellies@reed.edu

disease compared to those in clade 2, included several virulence factors: *rpoS*, 29 of the 41 locus of enterocyte effacement (LEE)-encoded genes, *gadX* involved in acid tolerance, and the pO157 plasmid-encoded *stcE* adhesin and *hlyA* hemolysin. These data provide evidence that genetic regulation correlates with EHEC virulence.

However, our understanding of the regulatory network controlling EHEC pathogenesis remains incomplete. Some of the questions pursued by researchers include the following: What are the molecular signals perceived by EHEC strains in the human host and within cattle that allow the bacteria to properly express virulence traits? What are the signals and bacterial responses required to pass through the acid environment of the stomach, to ultimately reside in the large intestine, and to cause disease in humans but to attach to and colonize harmlessly the recto-anal junction (RAJ) of cattle (5). How are expression of the type III secretion system (T3SS), attachment to host cell surfaces, the effector molecules destined for translocation into host cells, secretion itself, and the Shiga toxin leading to serious disease controlled? This article summarizes our current knowledge of EHEC virulence gene regulation, indicating that spatiotemporal control of pathogenesis in humans and carriage in cattle is coming to light.

## ACID TOLERANCE

As for any intestinal pathogen that causes disease, EHEC must survive the acidic environment of the stomach. Three acid resistance systems have been described: a glucose-repressed, or oxidative, system and glutamate- and arginine-dependent systems (for review see reference 6). Evidence suggests that the glucose-repressed system is used for EHEC survival in acidic food items (7). For the glutamate-dependent system, glutamate decarboxylases GadA and GadB convert glutamate to γ-amino butyric acid, or GABA (8–10). The arginine decarboxylase AdiA converts arginine to agmatine (11). The Gad system is regulated by a number of environmental conditions and the global regulatory proteins H-NS and CRP and alternate sigma factors RpoS and RpoN (8–10, 12, 13) (Fig. 1). For both amino acid-dependent systems, pH homeostasis is maintained by displacing the α-carboxyl group of the amino acids with a proton that is transported from the environment to the cytoplasm. Cytoplasmic glutamate and arginine are restored by their respective antiporters, GadC and AdiC. The glutamate-dependent Gad system provides a high level of acid tolerance and is necessary for passage through the stomachs and for colonization of the RAJ in cattle (7).

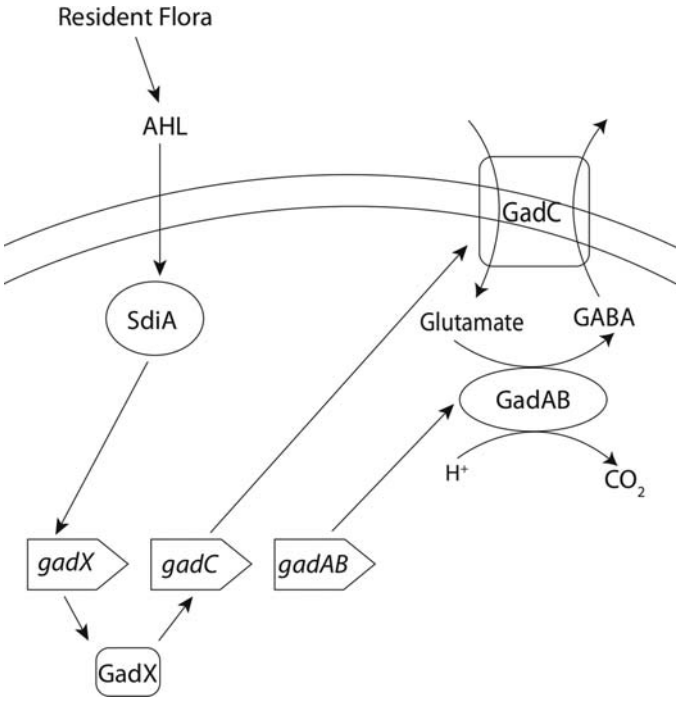



**FIGURE 1** Control of acid tolerance by EHEC. Resident flora produce AHL signaling molecules that stimulate expression of the glutamate-dependent acid resistance system (14). The GAD system removes excess protons by exchanging the alpha-carboxyl groups of glutamate with a proton. The resulting GABA molecules are transported out of the cell in exchange for additional glutamate (143). doi:10.1128/microbiolspec.EHEC-0004-2013.f1

In cattle, EHEC passes through the rumen to eventually colonize the RAJ, and the LuxR homolog SdiA is involved in this process. Although EHEC does not possess the LuxI *N*-acyl homoserine lactone (AHL) synthase, SdiA perceives the oxo-C6-homoserine lactone produced by other bacteria in the rumen (Fig. 1). In turn, SdiA increases *gadX* expression (14), which is a regulator of the *gad* genes, encoding glutamate-dependent acid resistance. Using a cattle model, investigators demonstrated that wild-type EHEC outcompeted the *sdiA* deletion strain, which was defective in colonization of the RAJ over the 6-day assay. The finding that an estimated 70 to 80% of cattle herds in the United States carry EHEC is a major step forward in our understanding of the infection process in the EHEC reservoir.

## MOTILITY

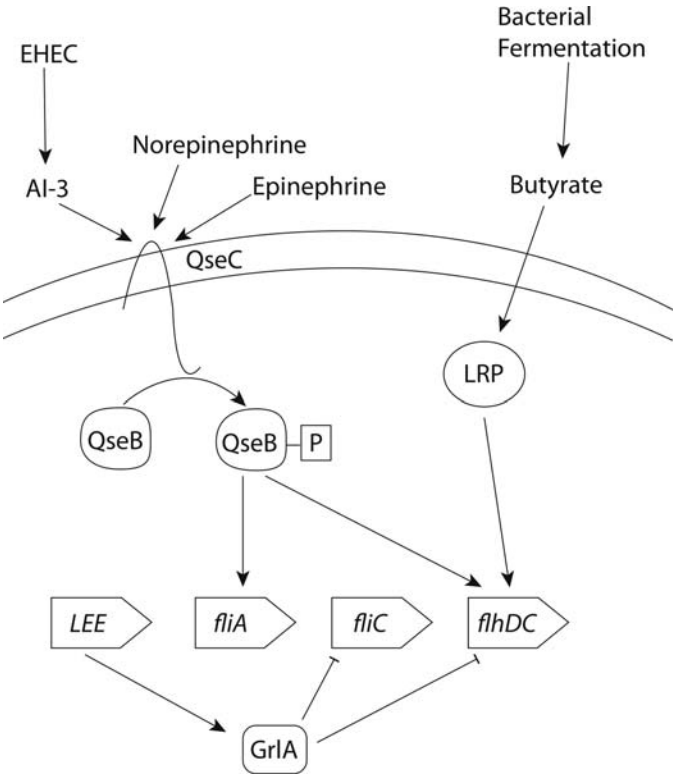
Flagellar motility is important in the initial stages of infection for many bacterial pathogens. For EHEC, motility and taxis are stimulated through the two-component system, QseC and QseB, which perceives

the AI-3 signal and the interkingdom communication molecules epinephrine and norepinephrine (15–18) (Fig. 2). QseBC signaling activates expression of the *flhDC* master regulators and FliA, an alternate sigma factor, ultimately turning on a number of operons necessary for flagellar biosynthesis and motility. Furthermore, motility and biofilm formation are enhanced in the presence of epinephrine and norepinephrine (19). It is important to note that the AI-3 quorum-sensing molecule is distinct from AI-2, which was initially thought to control motility and type III secretion (20–22). Unlike AI-2, AI-3 is not dependent on LuxS for synthesis. Deletion of the gene encoding the AI-3 and epinephrine- and norepinephrine-recognizing QseC sensor kinase attenuated EHEC virulence in a rabbit model of infection. Collectively, these data clearly demonstrate that flagellar motility, and the associated signaling, is necessary for virulence (15, 16).

Additional molecules are known to stimulate flagellar motility, and data indicate temporal control correlates with the infection process. Three classes of promoters are responsible for transcribing the flagellar operons (23). The *flhDC* operon is designated class I and is required for class II promoter activity, which includes the *fliA* operon. In turn, class II promoters are required for class III expression. Butyrate, a short-chain fatty acid found in the large intestine, increases expression of *flhDC* (24) (Fig. 2). Regulation of *flhDC* by butyrate is leucine-responsive regulatory protein (LRP)-dependent, whereas induction of *fliA* by this short-chain fatty acid is LRP-independent. This regulation does not occur in the presence of propionate or acetate. However, propionate and acetate do increase *fliC* (class III) expression but not through *flhDC*. Short-chain fatty acids are found in concentrations ranging from 20 to 140 mM in the large bowel, and recognition of this signal, along with the AI-3 and interkingdom signaling molecules, likely contributes to EHEC niche recognition and adaptation. Some evidence also suggests that the flagellum acts as an initial adhesin to epithelial cells from the bovine terminal rectum (25).

Q2

**FIGURE 2** Regulation of flagellar biosynthesis and motility. Extracellular signals norepinephrine, epinephrine, and AI-3 stimulate expression of *fliA* (motility) and *flhDC* (biosynthesis) through the two-component system QseBC (16). Following induction of the *LEE* operons, *grlA* inhibits expression of *fliC* and *flhDC* (28). Blunted arrow indicates negative control. doi:10.1128/microbiolspec.EHEC-0004-2013.f2




Once motility is no longer needed, or prior to its necessity for colonization, the flagella are downregulated, an important step toward avoiding immune recognition. One observed mechanism is that the LuxR homolog SdiA downregulates FliC expression (26). In another mechanism, mucin, produced by epithelial tissues, diminishes expression of flagellar genes. Mucin on agar plates repressed motility, and transcriptome analysis and quantitative PCR confirmed these data (27). In addition, the GrlA regulator, expressed from the LEE, decreased flagella biosynthesis by downregulating *fliC* and *flhDC* (28) (Fig. 2). Consistent with flagella not being needed after surface adherence, Tobe et al. observed that after 5 h of attachment to epithelial cells, flagella were downregulated in a GrlA-dependent manner. Furthermore, in a neonatal meningitis-causing *E. coli* strain, the *E. coli* common pilus (ECP), found in pathogens and nonpathogens alike, is necessary for attachment and biofilm formation. The regulator of ECP, EcpR, also downregulates flagellar motility after adherence occurs (29). By downregulating the flagellar master regulators *flhDC* by GrlA in EHEC, spatiotemporal regulation controls pathogenesis in coordination with the transition from a planktonic to an adhesive lifestyle.

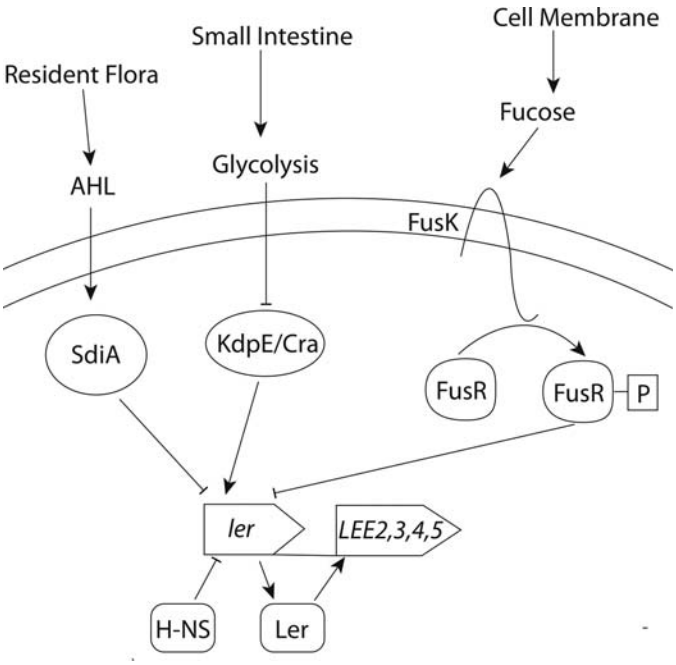
## CONTINUING THE INTESTINAL JOURNEY

Carbohydrate recognition and metabolism are important for EHEC niche adaptation (30) and ensure that EHEC virulence proteins are expressed only at the appropriate site of infection. A glycolytic environment, at

0.4% glucose, inhibits *ler* and LEE expression, whereas 0.1% glucose mimicking gluconeogenesis conditions enhances their expression (31). The LEE pathogenicity island (PAI) encodes T3SS and is organized into five major polycistronic operons and a number of bicistronic and monocistronic genes (32, 33). In gluconeogenesis, instead of oxidizing glucose, glucose is produced from two to three carbon molecules, such as acetate, succinate, or pyruvate. Consistently, glucose and glycerol inhibit *ler* expression, while succinate stimulates *ler*, mimicking glycolysis and gluconeogenesis, respectively (Fig. 3). This regulation occurs through the proteins KdpE, a response regulator that also senses osmotic stress, and Cra, both of which bind to *ler* regulatory DNA. Deletion of *kdpE* and *cra* results in ablation of attaching and effacing (A/E) lesion formation by EHEC in vitro(31).

In the gut, EHEC also perceives and responds to the sugar fucose, which enhances EHEC colonization (34). In the mammalian intestine, fucose is abundant, reaching concentrations of ~100 μM (35), because it is cleaved from host mucin glycoproteins by fucosidases produced by the commensal bacterium *Bacterioides thetaiotamicron*. EHEC perceives the fucose signal via the two-component regulatory pair FusK, the sensor kinase, and FusR, the response regulator (36) (Fig. 3). Transcriptional activity of the *LEE1* operon-encoded *ler* is increased in Δ*fusK* and Δ*fusR* strains. Thus these data indicate that the fucose-sensing, two-component system represses Ler and ultimately T3SS expression. Regulation is direct, as purified FusR binds to *ler* regulatory sequences, and binding is enhanced when the protein is in the phosphorylated state. This carbohydrate signaling is independent of using fucose as a carbon source. The importance of fucose signaling in virulence is highlighted by the findings that in an infant rabbit model of infection, *fusK* and *fusR* deletion mutants are outcompeted by the wild-type strain (36). In sum, *ler* and the T3SS are repressed by high glucose concentrations, and the presence of mucus-derived fucose, while stimulated under gluconeogenesis conditions, is mimicked by the presence of molecules such as succinate, pyruvate, acetate, and propionate.

**FIGURE 3** Inhibition of EHEC effector molecules in the cattle rumen and human small intestine. Resident flora in the rumen produce the signaling molecule AHL that causes SdiA to fold and inhibit transcription of *ler* (14). Glycolytic conditions in the small intestine inhibit the *ler* transcriptional activators KdpE and Cra (31). Fucose, a component of cell-surface glycans, signals through the two-component system FusKR to inhibit Ler expression (36). The transcriptional silencer H-NS maintains Ler downregulation (48). Blunted arrows indicate negative control. doi:10.1128/microbiolspec.EHEC-0004-2013.f3




EHEC virulence gene regulation has been correlated further with intestinal physiology. Butyrate, found in the large intestine, increases expression of the T3SS (37) (Fig. 4). More recently, ethanolamine, a bacterial and animal cell membrane component that is released into the lumen of the gut upon normal epithelial cell turnover, has been implicated as a molecule important in EHEC virulence gene regulation. Ethanolamine is perceived by EHEC, leading to increased expression of the quorum-sensing regulators QseA, QseC, and QseE and Ler (38). In addition, the *LEE* operons 1 through 5 are thus stimulated in the presence of ethanolamine. Though there are 17 ethanolamine utilization genes, *eut*, necessary for the use of this compound as a nitrogen source, the observed signaling in EHEC is independent of ethanolamine metabolism, but partially dependent on the EutR transcriptional regulator that binds ethanolamine (38) (Fig. 4). Furthermore, there is evidence that ethanolamine confers a growth advantage to EHEC during the stationary phase of growth, when this compound is used as a nitrogen source in media mimicking bovine intestinal contents (39). Ethanolamine is a part of normal physiology of the human and bovine large intestine membranes, and for EHEC, it provides a growth advantage and signals for expression of the T3SS as part of ecological niche recognition and adaptation. This signaling most likely coincides with close proximity or attachment to the host epithelium.

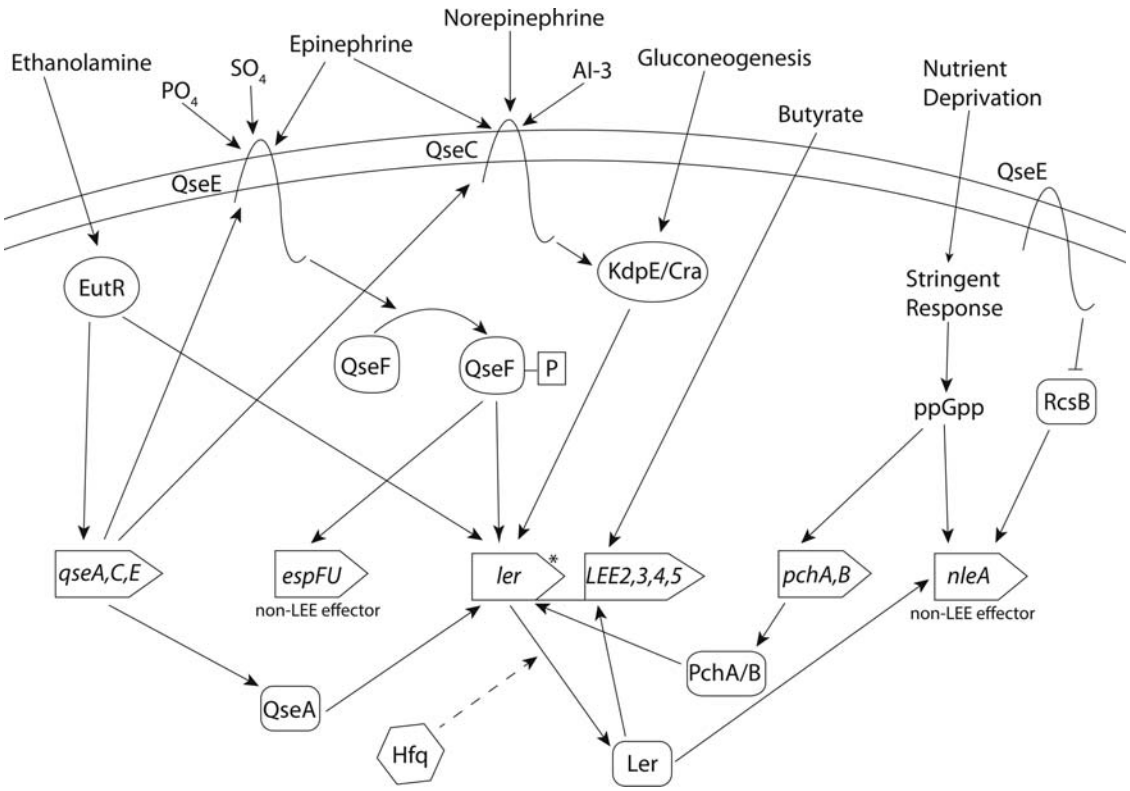



**FIGURE 4** Stimulation of LEE and non-LEE effector molecules required for infection by EHEC. In the large intestine of humans and RAJ, the host-produced hormones norepinephrine and epinephrine, EHEC-derived signaling molecule AI-3, and sulfate and phosphate trigger the two-component sensors QseC,F and/or QseEF to upregulate *ler* and *espFu* transcription (17, 85). In addition, ethanolamine, a cell membrane component, stimulates *ler* production through EutR and QseC (38). Gluconeogenic conditions of the large intestine activate the *ler* activators KdpE and Cra, and butyrate directly activates LEE transcriptional activity (37, 125). The RNA chaperone Hfq affects LEE expression through interactions with Ler mRNA but has negative or positive effects depending on the strain of EHEC, as indicated by a dashed arrow (71–73). Finally, nutrient deprivation associated with the infection site activates the stringent response leading to production of ppGpp, which promotes expression of the LEE transcriptional activators *pchA* and *pchB* and the non-LEE effector NleA (68, 117). The asterisk indicates that *ler* (*LEE1*) expression is upregulated by several other factors, including temperature, pH, iron, ammonium, calcium, bicarbonate, and the regulatory proteins IHF, Fis, BipA, PerC, and GadX (previously reviewed in reference 45). Blunted arrow indicates negative control. doi:10.1128/microbiolspec.EHEC-0004-2013.f4

## T3SS EXPRESSION AND ATTACHMENT

### LEE

Elaboration of the T3SS of EHEC allows for attachment to the intestinal epithelium in cattle, specifically to the epithelial cells of the RAJ (5, 40). Disruption or deletion of genes encoding components of this apparatus reduces colonization in the bovine host (41, 42). Furthermore, the translocated intimin receptor (Tir) and intimin proteins, facilitators of the tight attachment of the A/E intestinal lesions, play a critical role in colonization of a neonatal calf model of infection (43). In addition to binding to the bacterial-derived Tir molecule, intimin also binds to integrins and nucleolin on the host cell surface. The molecular syringe, the apparatus necessary for altering signaling events in the formation of actin-rich pedestals, ultimately injects ~50 distinct effector molecules into host cells (44). Assembly of the apparatus, expression of both LEE and non-LEE encoded effectors, and their translocation must be regulated in a spatiotemporally correct manner to establish infection and to avoid immune detection in both humans and cattle.

The LEE PAI, encoding the T3SS, is silenced by H-NS (for review see reference 45). Navarre et al. demonstrated that the function of H-NS, conserved across the *Enterobacteriaceae*, is to silence horizontally transferred genetic elements, indicated by a lower GC or higher AT content than the resident genome. Along with *LEE1*, multiple *LEE* operons are directly silenced by H-NS (46–48), and thus, much of the regulatory network described for the LEE directly involves relieving H-NS-mediated repression. The *LEE1*-encoded Ler protein, an H-NS homolog, is a key regulator of the LEE and acts as an antisilencer (32). As most H-NS-controlled genes are regulated in response to environmental adaptation (49), numerous environmental inputs and regulatory proteins control *ler* gene expression.

To demonstrate the many inputs controlling *ler* gene expression, researchers working with both EHEC and enteropathogenic *E. coli* (EPEC) found that Ler is stimulated in response to environmental signals such as temperature, pH, iron, ammonium, calcium, bicarbonate (50–54), and quorum-sensing signaling (55, 56), as well as the proteins IHF (57), Fis (58), BipA (59), PerC homologs, or Pch (60), GrlRA (61, 62), and GadX (63) (Fig. 4). The GrlRA regulators constitute a complicated feedback loop whereby GrlA directly activates *ler* expression, and GrlR acts posttranslationally to reduce the cellular quantity of the GrlA protein (28, 61, 64, 65). Once expressed, Ler, in turn, acts as an H-NS antisilencer to increase the expression of *LEE2*, *LEE3*, *LEE4,* and *LEE5* operons (Fig. 4).

LEE gene expression is stimulated as EHEC transitions into stationary phase, controlled by quorum-sensing signaling, and also in response to nutrient deprivation, much through direct regulation of *ler* (21) (Fig. 4). Nutrient deprivation, the starvation for a number of nutritional requirements such as amino acids, carbon, nitrogen, or phosphorus, induces the stringent response (for review see reference 66). The stringent response produces the signaling molecule ppGpp, a process dependent on the synthase RelA and SpoT, a hydrolase and synthase (67). Expression of the EHEC LEE and adherence to Caco-2 cells in culture are increased in response to nutrient deprivation in a RelA-dependent manner, whereby increased pools of ppGpp are created (68). The protein DksA, which interacts with RNA polymerase in the transcription complex, is also involved in this regulation. Regulation of EspB and Tir, and thus the *LEE4* and *LEE5* operons, is controlled, in part, by ppGpp interaction with the *ler*, *pchA,* and *pchB* genes. Thus the stringent response rapidly activates expression of the T3SS components when nutrients are limiting and upon transitioning into stationary phase, conditions EHEC is predicted to encounter when entering the lower intestine.

A number of posttranscriptional control mechanisms add to the complexity and highlight the importance of coordinating expression of LEE genes within the host. RNaseE-dependent RNA processing occurs at the *LEE4* operon, resulting in differential control of expression of the SepL protein. This RNA processing is thought to be involved in the contact regulation that controls the switch from translocator to effector type III secretion (62, 69) and the regulation of EspADB proteins necessary for forming the filament and pore that ultimately embed in the host cell membrane (70). In EHEC strain 86-24, serotype O157:H7, the RNA chaperone Hfq acts positively on the expression of *ler* and *LEE* operons 2 through 5 in all phases of growth (71) (Fig. 4). This study shows that Hfq increases the expression of the quorum-sensing, two-component system QseBC. QseC also acts to increase Ler expression and thus illustrates coordinated regulation. Other researchers, though working with strain EDL933 as opposed to 86-24, observed that the RNA chaperone Hfq negatively affects the expression of the LEE (72, 73). Shakhnovich et al. observed that Hfq negatively regulates expression of the LEE in a Ler-dependent manner and also controls expression of a number of non-LEE effector molecules. The *ler*, or *LEE1* transcript, was a direct target of Hfq regulation. Congruently, Hansen and Kaper observed that Hfq affects Ler expression by negatively affecting the GrlRA regulators through posttranscriptional control, the stability of the *grlRA* transcript, while negatively affecting LEE expression in stationary phase in a manner independent of *grlRA*. Consistently with this set of observations, researchers also demonstrated that Hfq negatively affects LEE expression in the related AE pathogen EPEC (73). Thus, LEE genes are apparently regulated by Hfq in an opposite manner in the O157:H7 strains 86-24 and EDL933. Collectively, these regulatory observations illustrate the intricate and variable nature of virulence gene control in related but distinct isolates associated with outbreaks of EHEC disease and the evolution of regulatory networks associated with niche adaptation.

Additionally, a number of O-island regulators contribute to the expression of the LEE. O-islands are horizontally acquired clusters of genes that reside in the EHEC genome. The regulators EtrA and EivF are encoded in a cryptic, second T3SS gene cluster in EHEC, and negatively regulate the LEE PAI and secretion (74). Encoded within O-island 51, the regulator RgdR is a

positive regulator of the LEE and secretion, through the Ler regulatory cascade (75). Thus expression of the T3SS is controlled not only by LEE-encoded regulators and regulatory elements that are part of the core *E. coli* genome but also by genes acquired by horizontal gene transfer.

PerC is an important regulator of the EPEC LEE, though a *perC*-like gene does not exist on the pO157 virulence plasmid of EHEC (33, 46, 76). However, in the O157:H7 Sakai stain, Iyoda and Watanabe (60) identified five chromosomally encoded PerC homologs (*pch*). Of those identified, *pchA*, *pchB*, and *pchC*, but not *pchD* or *pchE*, were confirmed to regulate the LEE when expressed in *trans*. Using double mutations, the authors demonstrated that, in combination, *pchA* and *pchB*, or *pchA* and *pchC* resulted in significantly decreased expression of the LEE, through the global regulator Ler, and reduction of adherence to HEp-2 epithelial cells in culture (Fig. 4). Interestingly, the *pchABC* genes of EHEC and *perC* of EPEC are interchangeable in their ability to activate expression of the *LEE1* operons of both organisms (77). Thus the EHEC *pch* genes, with the exception of *pchD and pchE*, all encoded within phage-like elements, are involved in pathogenesis.

Intense investigation has been directed toward cell-to-cell communication or quorum-sensing signaling of the LEE and its cognate effector molecules. The best understood of the autoinducers, AHLs, are not produced by *E. coli*. Thus EHEC strains do not have the LuxI AHL synthase, though they do possess a LuxR-type transcriptional regulator SdiA (78, 79), and structural studies indicate that SdiA properly folds only in the presence of AHLs (80). Therefore, regulation through SdiA occurs by perceiving AHLs produced by other bacteria, leading to alterations in EHEC gene expression.

AHL molecules are produced by the resident flora in the rumen of the bovine intestine, indicated by an *Agrobacterium* reporter strain, but are not found in the terminal rectum, the site of EHEC colonization (14). Through SdiA sensing of the oxo-C6-AHL signal LEE genes, including *ler* located in *LEE1* and *LEE4* operons, are downregulated (Fig. 3). It has been proposed that quorum sensing facilitates EHEC escaping the rumen en route to the site of colonization, the RAJ in cattle, by downregulation and avoiding inappropriate expression of the LEE, while inducing the *gad* acid tolerances system, as described above (Fig. 1).

Elucidation of the interkingdom signaling systems of EHEC controlling virulence has revealed how these organisms communicate with members of their own species, resident bacteria, and the host organism. The QseA quorum-sensing regulator acts directly on the expression of the *LEE1* operon, specifically at the P1 promoter (81, 82) (Fig. 4). Indeed, multiple two-component regulators perceive chemical signals and the EHEC-derived, aromatic AI-3 molecule to control expression of the T3SS. The sensor kinase QseE responds to sulfate, phosphate, and host-derived epinephrine to phosphorylate its cognate response regulator QseF that ultimately stimulates *ler* expression (83) (Fig. 4). The sensor kinase QseC perceives the catecholamines epinephrine and norepinephrine and the EHEC-produced AI-3 molecule to increase *ler* transcription through phosphorylation of KdpE (17), leading to the elaboration of the type III system. To add emphasis to the importance of quorum-sensing signaling controlling virulence, in an infant rabbit model of infection, deletion or inhibition of *qseC* in rabbit EPEC severely attenuated virulence (15, 84), and a *qseF* deletion mutant of EHEC does not form A/E lesions (85). Thus the ability to respond to host catecholamines and AI-3 is required for EHEC to stimulate T3SS expression and to establish infection.

## Non-LEE

A number of non-T3SS adhesins, either demonstrated to be necessary for or hypothesized to be involved in attachment to host cells, have been described (Table 1). Upon sequencing the *E. coli* O157:H7 genome of strain EDL933, it was noted that this organism has at least 10 fimbrial gene clusters and 13 regions that encode nonfimbrial adhesins, some of which were not found in nonpathogenic *E. coli* (86, 87). Extracellular structures include the *E. coli* YcbQ laminin-binding fimbriae (ELF) (88), two long polar fimbriae (LPF) (89, 90), the F9 fimbriae (91), a type IV pilus called "hemorrhagic *coli* pilus" (HCP) (92), curli (93, 94), OmpA (95), the EHEC factor for adherence (Efa1) (96), the IgrA homolog adhesin (Iha) (97), the ECP, the autotransporter protein EhaG (98), and the pO157 virulence plasmid-encoded StcE (99, 100). Surely these molecules contribute to adherence to surfaces, both biotic and abiotic, but a comprehensive understanding of the role of these adhesins in human disease, carriage in cattle, and survival and propagation in food does not exist.

However, the long polar fimbria 1 (Lpf1) of *E. coli* O157:H7 is a well-characterized example of one such adhesion. Lpf1 facilitates binding of EHEC to not only epithelial cells but also extracellular matrix proteins, including fibronectin, laminin, and collagen IV (101). Lpf1 is tightly regulated, with maximal expression in

**TABLE 1** Regulation of non-LEE adhesins and factors involved in EHEC adherence

| Adhesin | Type | Regulation | References |
|---|---|---|---|
| YcbQ | Laminin-binding fimbriae | | 88 |
| Lpf1 | Long-polar fimbriae | H-NS, Ler, late exponential phase, low pH | 89, 90, 101, 103–105, 146 |
| F9 | Fimbriae | | 91 |
| Curli | Fimbriae | Fis, Hha, RcsB, zinc, heat shock | 93, 94, 147–149 |
| OmpA | Outer membrane protein A | Hfq, nitrogen | 95, 150–152 |
| Efa1 | Toxin | | 96, 153 |
| Iha | IgrA homolog adhesin | Temperature, iron | 97, 154, 155 |
| ECP | *E. coli* common pilus | EcpR, H-NS, IHF | 106, 107 |
| EhaG | Trimeric autotransporter protein | H-NS | 98 |
| StcE | Zinc metalloprotease | H-NS, Ler | 99, 100, 108 |

late exponential phase of growth in iron-deprived and slightly acidic environments (102) (Table 1). Two *lpf* loci exist in *E. coli* O157:H7, and when one or both are deleted, colonization in animal infection models is altered (103, 104) (Table 1). The Lpf1 fimbria is coordinately regulated with the LEE because the operon is silenced by H-NS, while Ler functions as its antisilencer (105).

ECP, common to both pathogens and nonpathogens, has also been described. Deletion of the *ecp* genes resulted in decreased adherence of both O157:H7 strain EDL933 and commensal *E. coli* to human epithelial cells in culture (106). EcpR, a LuxR-like regulator containing a helix-turn-helix DNA-binding motif, controls expression of the ECP (107). The EcpR protein was shown to bind to a TTCCT sequence upstream of the *ecp* operon, and deletion of *ecpR* resulted in decreased adherence by EHEC. H-NS silences *ecp*, and antisilencing occurs by EcpR, assisted by the protein IHF. Thus the ECP is most likely involved in colonization by pathogens and nonpathogens alike. Similarly, a trimeric autotransporter protein, EhaG, of EHEC has been described (98). As for EcpR, these conserved proteins are found in both pathogens and nonpathogens, and in EHEC EhaG is involved in autoaggregation, biofilm formation, and adherence to extracellular matrix proteins and colorectal epithelial cells. Not surprisingly, expression is, in part, controlled by H-NS (Table 1). Finally, the StcE zinc metalloprotease encoded on the pO157 virulence plasmid enhances pedestal formation and is predicted to facilitate adherence to epithelial cells in the host by cleavage of glycoproteins (99, 108). The *stcE* gene is coordinately regulated with the LEE through Ler and H-NS. Clearly there is much work to be done to understand the role of non-T3SS adhesins in niche recognition, pathogenesis in general, and how their expression is coordinated with other virulence factors.

## INJECTION OF EFFECTORS

Effector molecules slated for injection by the T3SS into host cells are encoded within the LEE, O-islands, phage, and other integrative elements located in the genome. Those located within the LEE, including Tir, EspF, Map, EspG, EspH, and EspZ, are of course coordinately regulated with the expression of the secretion apparatus itself. Evidence demonstrates that a number of non-LEE-encoded effectors, including EspJ, NleB, NleE, NleF, and NleH, are involved in EHEC colonization and survival, though experiments demonstrating these phenotypes were conducted in a number of different animal infection models (109–111). Thus further experimentation is necessary to determine the role of these non-LEE effectors in EHEC disease and carriage, particularly in colonization of the RAJ in cattle.

Assuming that the non-LEE effectors are important for disease in humans and carriage in cattle, they should be coordinately expressed with LEE-encoded effectors. Investigators have begun to unravel their regulation. In particular, several studies have focused on the NleA (also named EspI) effector because of evidence that it plays an important role in pathogenesis. After translocation into the host cell, NleA colocalizes with the Golgi apparatus, and has been associated with severe disease in the *Citrobacter rodentium* A/E mouse model (112). NleA is conserved in many A/E pathogens (113, 114), and evidence indicates that it affects protein trafficking and secretion through the rough endoplasmic reticulum by binding to the COPII coat protein (112, 113). NleA is secreted into the EHEC extracellular milieu (115) and is coordinately regulated with the LEE. As for the LEE, the regulatory proteins H-NS, Ler, and GrlA control expression of the phage-encoded *nleA* gene (115–117). Ler acts directly, binding to the regulatory region upstream of the *nleA* promoter. As with *LEE1*, *nleA* is regulated by quorum-sensing signaling, whereby QseE controls expression through inhibition of the positive-acting RcsB response regulator (118). Expression of NleA is induced by osmolarity, response to starvation signals, and RecA-dependent DNA-damage signaling, the latter also controlling LEE expression (117, 119) (Fig. 4).

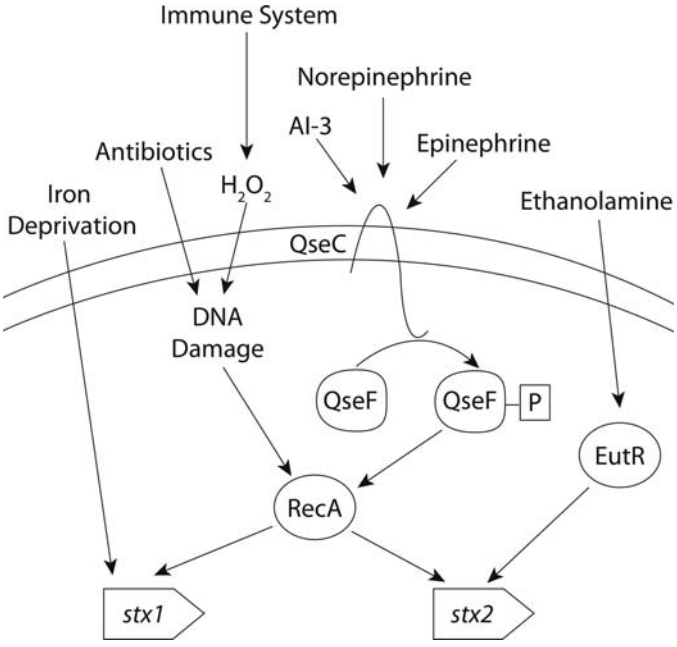
EspFu (also named TccP) is another non-LEE effector important for the EHEC A/E histopathology. Unlike EPEC where all genes required for A/E lesion formation are found within the LEE, EspFu is necessary for this phenotype in EHEC and is encoded within phage U (120–122). EspFu binds to the GTP-binding domain of N-WASP, mimicking the eukaryotic SH2/SH3 adapter protein and facilitates actin polymerization, and is subject to regulation by environmental signals (123). The genes *espFu* and *espJ* are influenced by changes in temperature, pH, osmolarity, and oxygen pressure (124). The regulators KdpE and Cra act to increase expression of *espFu* (125). The QseEF two-component, quorum-sensing system controls expression of EspFu through AI-3, epinephrine, and norepinephrine signaling (85) (Fig. 4). Thus the non-LEE effectors NleA and EspFu, important for virulence in EHEC, are coordinately controlled with the LEE by overlapping signaling: *nleA* expression is Ler-dependent and, in part, induced by the SOS response, while expression of *espFu* is controlled by the QseA and QseEF quorum-sensing pathways that directly stimulate the LEE (Fig. 4).

Data are emerging to show that there are at least two modes of regulation of the non-LEE effector molecules. The study by Garcia-Angulo et al. (116), addressing whether a common mechanism for the non-LEE effector molecules exists, is an important investigation because to date a common regulatory network linking their expression had not been established. Though performing much of their experimentation on the related A/E pathogen EPEC, investigators identified a 13-bp inverted repeat, with a 5-bp spacer upstream of many of the genes encoding the non-LEE effector molecules. They found in EPEC, by deletion of these sequences and mutagenesis, that the repeats were essential for transcription of the *nleH1* and *nleB2* genes. The authors termed these sequences NRIR, for *nle* regulatory inverted repeat. Transferring this information to EHEC virulence gene regulation, the authors found a number of *nle* genes in the EDL933 and Sakai EHEC strains that contain NRIR sequences in positions predicted to affect transcription by in silico analysis. These loci included, in both strains EDL933 and Sakai, *nleH1-nleF1*, *nleB2-nleC*, *espX*, a number of *nleG* genes, and *espFu*. The authors concluded that the NRIR sequences were involved in coordinated expression of the non-LEE effector molecules in a second mode of regulation, independent of the LEE regulators Ler and GrlA. Future work will undoubtedly involve identifying protein components, as well as additional genetic elements necessary for this regulatory network, and determining how these effector molecules are coordinately regulated with the type III system required for their translocation into host cells.

## TOXIN EXPRESSION AND PHAGE INDUCTION

As with many of the non-LEE effector molecules and most major bacterial exotoxins, the EHEC *stx1* and *stx2* genes are encoded within prophages. The Shiga toxins Stx1 and Stx2 are responsible for bloody diarrhea associated with EHEC infection and the serious complication hemolytic-uremic syndrome. Toxin production occurs upon phage induction. The genes *stx1* and *stx2* are encoded in separate prophages, and both are expressed during the phage lytic cycle (126). Initial studies indicated that *stx1* is also regulated by low levels of iron as a mechanism to acquire this micronutrient (127–129) (Fig. 5). As for induction of other lambdoid phage, Stx production is induced by DNA damage and regulated by RecA. Antibiotics that target DNA synthesis, such as quinolones and mitomycin C, stimulate Stx production

**FIGURE 5** Stimulation of Shiga toxin expression. Low iron levels lead to upregulation of *stx1* (127, 128). Hydrogen peroxide and antibiotics targeting DNA synthesis, such as mytomycin C and quinolines, lead to DNA damage and activate RecA to induce Stx1 production (130, 144, 145). Sulfate, phosphate, and epinephrine molecules signal through the two-component system QseCF to RecA, thus leading activation of Stx2 production. Ethanolamine also upregulates *stx2* through the transcription factor EutR (38). doi:10.1128/microbiolspec.EHEC-0004-2013.f5



(130). Similarly, hydrogen peroxide induces *stx* expression, most likely due to activation of the SOS response (131). In a seemingly contradictory study, the reactive oxygen species NO inhibited Stx phage induction and Stx production in the presence of the DNA-damaging agent mitomycin C (132). Here the authors claim that iNOS expressed in enterocytes, simulated by type I cytokines producing NO, leads to a bacterial NsrR-related stress response, sensitizing and protecting EHEC from DNA damage. Nonetheless, because the only known mechanism of Stx release is by lysing of the bacterium, combined with the now obvious reasons that DNA-damaging antibiotics lead to increased risk of hemolytic-uremic syndrome, there is a need for a more complete understanding of how *stx* genes are regulated.

More recent data correlate Stx production with signal molecules associated with specific regions of the human and bovine intestine. The two-component sensor kinase QseC regulates Stx2 expression through the SOS response, RecA cleavage of the lambda repressor cI (17) (Fig. 5). Consistently, *recA* expression was decreased in a *qseC* mutant. QseC responds to the interkingdom signaling molecules epinephrine and norepinephrine and the related AI-3 quorum-sensing molecule produced by *E. coli*. Several studies indicated that Stx production is controlled by AI-3. Another two-component pair, QseE, the sensor kinase, and QseF, the response regulator, affects Stx expression (85, 133). QseF can be phosphorylated by either QseE, responding to epinephrine, sulfate, and phosphate, or QseC to stimulate *stx2*. Ethanolamine, released from epithelium, also stimulates Stx2 production in EHEC (38). The genetic pathway necessary for the signaling involves the ethanolamine utilization regulator *eutR*, as deletion of the gene encoding this protein resulted in decreased expression of *stx2* by microarray (Fig. 5). Thus we begin to see a clearer picture of Stx regulation in EHEC, correlating expression to bacteria-derived autoinducers, host-produced signal molecules, and stress-related signals, and ultimately the genetic pathways necessary for the bacterial response.

Because of the necessity of iron for colonization and pathogenesis within the animal host, the EhxA enterohemolysin is also an important EHEC virulence factor. EhxA is a member of the RTX family, and its function is to lyse red blood cells. The *ehxCABD* operon, found on the pO157 virulence plasmid (134), is positively regulated by DsrA and RpoS at host body temperature (135). DsrA is a small RNA that acts by allowing translation to proceed (136). The expression of *ehx* is also positively regulated by GrlA and Ler (64, 137), and thus the enterohemolysin is coordinately regulated with the T3SS.

## A REGULATED INFECTION CONTINUUM

The work of many research groups leads to a model of EHEC pathogenesis correlating with gut physiology, bacterial metabolism along the route of infection, and host-microbe and microbe-microbe signaling. For both the human and bovine hosts, EHEC must pass through the acidic environment of the stomach and the rumen, respectively. Three acid tolerance systems assist the bacterium in this process. GadX is induced by AHL molecules produced by resident flora of the bovine rumen (14) and perceived by the LuxR homolog SdiA (Fig. 1). The LEE is downregulated by SdiA in this environment because it is not the normal site of attachment, allowing the bacterium to travel toward the RAJ in cattle (Fig. 3). Consistently, in a separate study, data indicated that flagellar motility is upregulated by acid stress while expression of Stx remains unchanged and the T3SS is downregulated by acute acid stress (138).

In the large intestines of humans, conditions mimicking gluconeogenic versus glycolytic metabolism are predicted to stimulate expression of the EHEC T3SS (31, 139) (Fig. 4). This observation is also understandable in colonization of the RAJ of cattle, where nutrients, particularly carbohydrates, are less abundant compared to the rumen. One might posit that EHEC avoids the small intestine in humans by downregulating expression of LEE genes in the presence of high-glucose, glycolytic conditions (Fig. 3). It is curious why EPEC, which infects the small intestine, also downregulates LEE genes under glycolytic conditions, whereas, identical to EHEC, LEE genes are stimulated under low-glucose conditions. This observation might be explained by the specificity of the infection process, that perception of multiple signals is necessary for proper niche recognition.

Carbohydrate metabolism and signaling are clearly linked to virulence, and several studies have indicated that fucose is important for EHEC colonization (34, 140). Resident *Bacterioides* spp. cleave fucose from the glycans found in the intestine, and suppression of Ler, T3SS function, and A/E lesions ensues from the fucose signal perceived by FusKR (36) (Fig. 3). EHEC can use a number of mucus-derived carbohydrates, in particular, mannose, *N*-acetylglucosamine, and *N*-acetylglucosamine, and galactose catabolism confers a competitive advantage against commensal bacteria, most likely assisting safe passage through the small intestine (39, 141). In both

humans and cattle, EHEC expresses the T3SS in response to membrane-derived ethanolamine, clearly encountered at the sites of infection in both hosts. The catecholamine and AI-3 signals also contribute to elaboration of the secretion apparatus (Fig. 4). Why would epinephrine and norepinephrine exist here when the adrenergic receptors are not located on the apical side of the intestinal lumen, but these molecules were detected in the lumen of the intestine of mice containing specific pathogen-free microbiota (142). It is also plausible that the epinephrine/norepinephrine signal becomes more available upon damage to the host epithelium, releasing these hormones into the lumen of the gut, enhancing expression of the T3SS and firmly establishing infection (Fig. 4). Nonetheless, researchers have established that three important signals, ethanolamine, epinephrine/norepinephrine, combined with the bacteria-derived AI-3, correlated with the sites of infection.

A number of signals, including the catecholamines, also control the expression of *stx* genes, mediated through two-component, quorum-sensing proteins QseC and QseE (Fig. 5). Membrane-derived ethanolamine and the well-studied RecA-dependent SOS response stimulate Stx production. EHEC is expected to encounter all of these conditions during niche recognition at the sites of attachment in humans and cattle. These observations are consistent with the presumption that substantive expression of Stx will occur when colonization has commenced.

## CONCLUSIONS

Researchers have made significant progress toward understanding the genetic regulation controlling EHEC pathogenesis. How EHEC gains safe passage through the acidic environment of the stomach, how flagellar motility is turned on and turned off, and what signals and metabolites are necessary for outcompeting commensal flora prior to arriving at the sites of infection for humans and cattle are now recognized on a basic level. Researchers have discovered myriad regulatory networks and environmental signals that control type III secretion, and we are beginning to understand how effector molecules slated for translocation into host cells are coordinately regulated with the T3SS itself and how the dangerous Shiga toxin is expressed. Though the LEE-encoded effectors and non-LEE NleA and EspFU are controlled by Ler and quorum-sensing signaling, the majority of those proteins targeted for secretion are regulated by different mechanisms. The NRIR sequences found upstream of many of the phage- and O-island-encoded effectors most likely coordinate their expression, but this has yet to be established experimentally for EHEC. Similarly, with the exception of Lpf1 and StcE, we do not know if, or how, expression of most non-LEE adhesins is coordinated with that of the LEE (Table 1). Finally, EHEC tissue tropism is not well understood, but the regulatory networks described herein most likely play an important role in niche recognition in human and animal hosts.

## REFERENCES


**1. Lukjancenko O, Wassenaar TM, Ussery DW.** 2010. Comparison of 61 sequenced *Escherichia coli* genomes. *Microb Ecol* **60:**708–720.

**2. Zhaxybayeva O, Doolittle WF.** 2011. Lateral gene transfer. *Curr Biol* **21:**R242–R246.

**3. Abu-Ali GS, Ouellette LM, Henderson ST, Lacher DW, Riordan JT, Whittam TS, Manning SD.** 2010. Increased adherence and expression of virulence genes in a lineage of *Escherichia coli* O157:H7 commonly associated with human infections. *PLoS ONE* **5:**e10167.

**4. Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, Mladonicky JM, Somsel P, Rudrik JT, Dietrich SE, Zhang W, Swaminathan B, Alland D, Whittam TS.** 2008. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. *Proc Natl Acad Sci USA* **105:**4868–4873.

**5. Naylor SW, Low JC, Besser TE, Mahajan A, Gunn GJ, Pearce MC, McKendrick IJ, Smith DG, Gally DL.** 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect Immun* **71:**1505–1512.

**6. Foster JW.** 2004. *Escherichia coli* acid resistance: tales of an amateur acidophile. *Nat Rev Microbiol* **2:**898–907.

**7. Price SB, Wright JC, DeGraves FJ, Castanie-Cornet MP, Foster JW.** 2004. Acid resistance systems required for survival of *Escherichia coli* O157:H7 in the bovine gastrointestinal tract and in apple cider are different. *Appl Environ Microbiol* **70:**4792–4799.

**8. Castanie-Cornet MP, Penfound TA, Smith D, Elliott JF, Foster JW.** 1999. Control of acid resistance in *Escherichia coli*. *J Bacteriol* **181:**3525–3535.

**9. De Biase D, Tramonti A, Bossa F, Visca P.** 1999. The response to stationary-phase stress conditions in *Escherichia coli*: role and regulation of the glutamic acid decarboxylase system. *Mol Microbiol* **32:**1198–1211.

**10. Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, Foster JW.** 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl Environ Microbiol* **62:**3094–3100.

**11. Gong S, Richard H, Foster JW.** 2003. YjdE (AdiC) is the arginine: agmatine antiporter essential for arginine-dependent acid resistance in *Escherichia coli*. *J Bacteriol* **185:**4402–4409.

**12. Mitra A, Fay PA, Morgan JK, Vendura KW, Versaggi SL, Riordan JT.** 2012. Sigma factor N, liaison to an ntrC and rpoS dependent regulatory pathway controlling acid resistance and the LEE in enterohemorrhagic *Escherichia coli*. *PLoS ONE* **7:**e46288.

**13. Tramonti A, Visca P, De Canio M, Falconi M, De Biase D.** 2002. Functional characterization and regulation of gadX, a gene encoding an AraC/XylS-like transcriptional activator of the *Escherichia coli* glutamic acid decarboxylase system. *J Bacteriol* **184:**2603–2613.

**14. Hughes DT, Terekhova DA, Liou L, Hovde CJ, Sahl JW, Patankar AV, Gonzalez JE, Edrington TS, Rasko DA, Sperandio V.** 2010. Chemical sensing in mammalian host-bacterial commensal associations. *Proc Natl Acad Sci USA* **107:**9831–9836.

**15. Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V.** 2006. The QseC sensor kinase: a bacterial adrenergic receptor. *Proc Natl Acad Sci USA* **103:**10420–10425.

**16. Clarke MB, Sperandio V.** 2005. Transcriptional regulation of flhDC by QseBC and sigma (FliA) in enterohaemorrhagic *Escherichia coli*. *Mol Microbiol* **57:**1734–1749.

**17. Hughes DT, Clarke MB, Yamamoto K, Rasko DA, Sperandio V.** 2009. The QseC adrenergic signaling cascade in enterohemorrhagic *E. coli* (EHEC). *PLoS Pathog* **5:**e1000553.

**18. Sperandio V, Torres AG, Kaper JB.** 2002. Quorum sensing *Escherichia coli* regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. *Mol Microbiol* **43:**809–821.

**19. Bansal T, EnglertD, Lee J, Hegde M, Wood TK, Jayaraman A.** 2007. Differential effects of epinephrine, norepinephrine, and indole on *Escherichia coli* O157:H7 chemotaxis, colonization, and gene expression. *Infect Immun* **75:**4597–4607.

**20. Kendall MM, Rasko DA, Sperandio V.** 2007. Global effects of the cell-to-cell signaling molecules autoinducer-2, autoinducer-3, and epinephrine in a luxS mutant of enterohemorrhagic *Escherichia coli*. *Infect Immun* **75:**4875–4884.

**21. Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB.** 2003. Bacteria-host communication: the language of hormones. *Proc Natl Acad Sci USA* **100:**8951–8956.

**22. Walters M, Sircili MP, Sperandio V.** 2006. AI-3 synthesis is not dependent on luxS in *Escherichia coli*. *J Bacteriol* **188:**5668–5681.

**23. Kutsukake K, Ohya Y, Iino T.** 1990. Transcriptional analysis of the flagellar regulon of *Salmonella typhimurium*. *J Bacteriol* **172:**741–747.

**24. Tobe T, Nakanishi N, Sugimoto N.** 2011. Activation of motility by sensing short-chain fatty acids via two steps in a flagellar gene regulatory cascade in enterohemorrhagic *Escherichia coli*. *Infect Immun* **79:**1016–1024.

**25. Mahajan A, Currie CG, Mackie S, Tree J, McAteer S, McKendrick I, McNeilly TN, Roe A, La Ragione RM, Woodward MJ, Gally DL, Smith DG.** 2009. An investigation of the expression and adhesin function of H7 flagella in the interaction of *Escherichia coli* O157:H7 with bovine intestinal epithelium. *Cell Microbiol* **11:**121–137.

**26. Sharma VK, Bearson SM, Bearson BL.** 2010. Evaluation of the effects of sdiA, a luxR homologue, on adherence and motility of *Escherichia coli* O157:H7. *Microbiology* **156:**1303–1312.

**27. Kim JC, Yoon JW, Kim CH, Park MS, Cho SH.** 2012. Repression of flagella motility in enterohemorrhagic *Escherichia coli* O157:H7 by mucin components. *Biochem Biophys Res Commun* **423:**789–792.

**28. Iyoda S, Koizumi N, Satou H, Lu Y, Saitoh T, Ohnishi M, Watanabe H.** 2006. The GrlR-GrlA regulatory system coordinately controls the expression of flagellar and LEE-encoded type III protein secretion systems in enterohemorrhagic *Escherichia coli*. *J Bacteriol* **188:**5682–5692.

**29. Lehti TA, Bauchart P, Dobrindt U, Korhonen TK, Westerlund-Wikstrom B.** 2012. The fimbriae activator MatA switches off motility in *Escherichia coli* by repression of the flagellar master operon flhDC. *Microbiology* **158:**1444–1455.

**30. Miranda RL, Conway T, Leatham MP, Chang DE, Norris WE, Allen JH, Stevenson SJ, Laux DC, Cohen PS.** 2004. Glycolytic and gluconeogenic growth of *Escherichia coli* O157:H7 (EDL933) and *E. coli* K-12 (MG1655) in the mouse intestine. *Infect Immun* **72:**1666–1676.

**31. Njoroge JW, Nguyen Y, Curtis MM, Moreira CG, Sperandio V.** 2012. Virulence meets metabolism: Cra and KdpE gene regulation in enterohemorrhagic *Escherichia coli*. *MBio* **3:**e00280-12.

**32. Elliott SJ, Sperandio V, Giron JA, Shin S, Mellies JL, Wainwright L, Hutcheson SW, McDaniel TK, Kaper JB.** 2000. The locus of enterocyte effacement (LEE)-encoded regulator controls expression of both LEE- and non-LEE-encoded virulence factors in enteropathogenic and enterohemorrhagic *Escherichia coli*. *Infect Immun* **68:**6115–6126.

**33. Mellies JL, Elliott SJ, Sperandio V, Donnenberg MS, Kaper JB.** 1999. The Per regulon of enteropathogenic *Escherichia coli*: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Ler). *Mol Microbiol* **33:**296–306.

**34. Snider TA, Fabich AJ, Conway T, Clinkenbeard KD.** 2009. *E. coli* O157:H7 catabolism of intestinal mucin-derived carbohydrates and colonization. *Vet Microbiol* **136:**150–154.

**35. Jaswal VM, Babbar HS, Mahmood A.** 1988. Changes in sialic acid and fucose contents of enterocytes across the crypt-villus axis in developing rat intestine. *Biochem Med Metab Biol* **39:**105–110.

**36. Pacheco AR, Curtis MM, Ritchie JM, Munera D, Waldor MK, Moreira CG, Sperandio V.** 2012. Fucose sensing regulates bacterial intestinal colonization. *Nature* **492:**113–117.

**37. Nakanishi N, Tashiro K, Kuhara S, Hayashi T, Sugimoto N, Tobe T.** 2009. Regulation of virulence by butyrate sensing in enterohaemorrhagic *Escherichia coli*. *Microbiology* **155:**521–530.

**38. Kendall MM, Gruber CC, Parker CT, Sperandio V.** 2012. Ethanolamine controls expression of genes encoding components involved in interkingdom signaling and virulence in enterohemorrhagic *Escherichia coli* O157:H7. *MBio* **3:**e00050-12.

**39. Bertin Y, Girardeau JP, Chaucheyras-Durand F, Lyan B, Pujos-Guillot E, Harel J, Martin C.** 2011. Enterohaemorrhagic *Escherichia coli* gains a competitive advantage by using ethanolamine as a nitrogen source in the bovine intestinal content. *Environ Microbiol* **13:**365–377.

**40. Low JC, McKendrick IJ, McKechnie C, Fenlon D, Naylor SW, Currie C, Smith DG, Allison L, Gally DL.** 2005. Rectal carriage of enterohemorrhagic *Escherichia col*i O157 in slaughtered cattle. *Appl Environ Microbiol* **71:**93–97.

**41. Dziva F, van Diemen PM, Stevens MP, Smith AJ, Wallis TS.** 2004. Identification of *Escherichia coli* O157:H7 genes influencing colonization of the bovine gastrointestinal tract using signature-tagged mutagenesis. *Microbiology* **150:**3631–3645.

**42. Naylor SW, Roe AJ, Nart P, Spears K, Smith DG, Low JC, Gally DL.** 2005. *Escherichia coli* O157:H7 forms attaching and effacing lesions at the terminal rectum of cattle and colonization requires the LEE4 operon. *Microbiology* **151:**2773–2781.

**43. Dean-Nystrom EA, Bosworth BT, Moon HW, O'Brien AD.** 1998. *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. *Infect Immun* **66:**4560–4563.

**44. Tobe T, Beatson SA, Taniguchi H, Abe H, Bailey CM, Fivian A, Younis R, Matthews S, Marches O, Frankel G, Hayashi T, Pallen MJ.** 2006. An extensive repertoire of type III secretion effectors in *Escherichia coli* O157 and the role of lambdoid phages in their dissemination. *Proc Natl Acad Sci USA* **103:**14941–14946.

**45. Mellies JL, ABarron AM, Carmona AM.** 2007. Enteropathogenic and enterohemorrhagic *Escherichia coli* virulence gene regulation. *Infect Immun* **75:**4199–4210.

**46. Bustamante VH, Santana FJ, Calva E, Puente JL.** 2001. Transcriptional regulation of type III secretion genes in enteropathogenic *Escherichia coli:* Ler antagonizes H-NS-dependent repression. *Mol Microbiol* **39:**664–678.

**47. Haack KR, Robinson CL, Miller KJ, Fowlkes JW, Mellies JL.** 2003. Interaction of Ler at the *LEE5* (*tir*) operon of enteropathogenic *Escherichia coli*. *Infect Immun* **71:**384–392.

**48. Umanski T, Rosenshine I, Friedberg D.** 2002. Thermoregulated expression of virulence genes in enteropathogenic *Escherichia coli*. *Microbiology* **148:**2735–2744.

**49. Hommais F, Krin E, Laurent-Winter C, Soutourina O, Malpertuy A, Le Caer JP, Danchin A, Bertin P.** 2001. Large-scale monitoring of pleiotropic regulation of gene expression by the prokaryotic nucleoid-associated protein, H-NS. *Mol Microbiol* **40:**20–36.

**50. Abe H, Tatsuno I, Tobe T, Okutani A, Sasakawa C.** 2002. Bicarbonate ion stimulates the expression of locus of enterocyte effacement-

encoded genes in enterohemorrhagic *Escherichia coli* O157:H7. *Infect Immun* **70:**3500–3509.

**51. Beltrametti F, Kresse AU, Guzman CA.** 1999. Transcriptional regulation of the *esp* genes of enterohemorrhagic *Escherichia coli*. *J Bacteriol* **181:**3409–3418.

**52. Ide T, Michgehl S, Knappstein S, Heusipp G, Schmidt MA.** 2003. Differential modulation by Ca2+ of type III secretion of diffusely adhering enteropathogenic *Escherichia coli*. *Infect Immun* **71:**1725–1732.

**53. Kenny B, Abe A, Stein M, Finlay BB.** 1997. Enteropathogenic *Escherichia coli* protein secretion is induced in response to conditions similar to those in the gastrointestinal tract. *Infect Immun* **65:**2606–2612.

**54. Kenny B, Finlay BB.** 1995. Protein secretion by enteropathogenic *Escherichia coli* is essential for transducing signals to epithelial cells. *Proc Natl Acad Sci USA* **92:**7991–7995.

**55. Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB.** 1999. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl AcadSci USA* **96:**15196–15201.

**56. Sperandio V, Torres AG, Giron JA, Kaper JB.** 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J Bacteriol* **183:**5187–5197.

**57. Friedberg D, Umanski T, Fang Y, Rosenshine I.** 1999. Hierarchy in the expression of the locus of enterocyte effacement genes of enteropathogenic *Escherichia coli*. *Mol Microbiol* **34:**941–952.

**58. Goldberg MD, Johnson M, Hinton JC, Williams PH.** 2001. Role of the nucleoid-associated protein Fis in the regulation of virulence properties of enteropathogenic *Escherichia coli*. *Mol Microbiol* **41:**549–559.

**59. Grant AJ, Farris M, Alefounder P, Williams PH, Woodward MJ, O'Connor CD.** 2003. Co-ordination of pathogenicity island expression by the BipA GTPase in enteropathogenic *Escherichia coli* (EPEC). *Mol Microbiol* **48:**507–521.

**60. Iyoda S, Watanabe H.** 2004. Positive effects of multiple *pch* genes on expression of the locus of enterocyte effacement genes and adherence of enterohaemorrhagic *Escherichia coli* O157:H7 to HEp-2 cells. *Microbiology* **150:**2357–2571.

**61. Barba J, Bustamante VH, Flores-Valdez MA, Deng W, Finlay BB, Puente JL.** 2005. A positive regulatory loop controls expression of the locus of enterocyte effacement-encoded regulators Ler and GrlA. *J Bacteriol* **187:**7918–7930.

**62. Deng W, Puente JL, Gruenheid S, Li Y, Vallance BA, Vazquez A, Barba J, Ibarra JA, O'Donnell P, Metalnikov P, Ashman K, Lee S, Goode D, Pawson T, Finlay BB.** 2004. Dissecting virulence: systematic and functional analyses of a pathogenicity island. *Proc Natl Acad Sci USA* **101:**3597–3602.

**63. Shin S, Castanie-Cornet MP, Foster JW, Crawford JA, Brinkley C, Kaper JB.** 2001. An activator of glutamate decarboxylase genes regulates the expression of enteropathogenic *Escherichia coli* virulence genes through control of the plasmid-encoded regulator, Per. *Mol Microbiol* **41:**1133–1150.

**64. Iyoda S, Honda N, Saitoh T, Shimuta K, Terajima J, Watanabe H, Ohnishi M.** 2011. Coordinate control of the locus of enterocyte effacement and enterohemolysin genes by multiple common virulence regulators in enterohemorrhagic *Escherichia coli*. *Infect Immun* **79:**4628–4637.

**65. Russell RM, Sharp FC, Rasko DA, Sperandio V.** 2007. QseA and GrlR/GrlA regulation of the locus of enterocyte effacement genes in enterohemorrhagic *Escherichia coli*. *J Bacteriol* **189:**5387–5392.

**66. Boutte CC, Crosson S.** 2013. Bacterial lifestyle shapes stringent response activation. *Trends Microbiol* **21:**174–180.

**67. Hernandez VJ, Bremer H.** 1991. *Escherichia coli* ppGpp synthetase II activity requires spoT. *J Biol Chem* **266:**5991–5999.

**68. Nakanishi N, Abe H, Ogura Y, Hayashi T, Tashiro K, Kuhara S, Sugimoto N, Tobe T.** 2006. ppGpp with DksA controls gene expression in the locus of enterocyte effacement (LEE) pathogenicity island of enterohaemorrhagic *Escherichia coli* through activation of two virulence regulatory genes. *Mol Microbiol* **61:**194–205.

**69. Bhatt S, Edwards AN, Nguyen HT, Merlin D, Romeo T, Kalman D.** 2009. The RNA binding protein CsrA is a pleiotropic regulator of the locus of enterocyte effacement pathogenicity island of enteropathogenic *Escherichia coli*. *Infect Immun* **77:**3552–3568.

**70. Lodato PB, Kaper JB.** 2009. Post-transcriptional processing of the LEE4 operon in enterohaemorrhagic *Escherichia coli*. *Mol Microbiol* **71:**273–290.

**71. Kendall MM, Gruber CC, Rasko DA, Hughes DT, Sperandio V.** 2011. Hfq virulence regulation in enterohemorrhagic *Escherichia coli* O157:H7 strain 86-24. *J Bacteriol* **193:**6843–6851.

**72. Hansen AM, Kaper JB.** 2009. Hfq affects the expression of the LEE pathogenicity island in enterohaemorrhagic *Escherichia coli*. *Mol Microbiol* **73:**446–465.

**73. Shakhnovich EA, Davis BM, Waldor MK.** 2009. Hfq negatively regulates type III secretion in EHEC and several other pathogens. *Mol Microbiol* **74:**347–363.

**74. Zhang L, Chaudhuri RR, Constantinidou C, Hobman JL, Patel MD, Jones AC, Sarti D, Roe AJ, Vlisidou I, Shaw RK, Falciani F, Stevens MP, Gally DL, Knutton S, Frankel G, Penn CW, Pallen MJ.** 2004. Regulators encoded in the *Escherichia coli* type III secretion system 2 gene cluster influence expression of genes within the locus for enterocyte effacement in enterohemorrhagic E. coli O157:H7. *Infect Immun* **72:**7282–7293.

**75. Flockhart AF, Tree JJ, Xu X, Karpiyevich M, McAteer SP, Rosenblum R, Shaw DJ, Low CJ, Best A, Gannon V, Laing C, Murphy KC, Leong JM, Schneiders T, La Ragione R, Gally DL.** 2012. Identification of a novel prophage regulator in *Escherichia coli* controlling the expression of type III secretion. *Mol Microbiol* **83:**208–223.

**76. Gomez-Duarte OG, Kaper JB.** 1995. A plasmid-encoded regulatory region activates chromosomal eaeA expression in enteropathogenic *Escherichia coli*. *Infect Immun* **63:**1767–1776.

**77. Porter ME, Mitchell P, Free A, Smith DG, Gally DL.** 2005. The *LEE1* promoters from both enteropathogenic and enterohemorrhagic *Escherichia coli* can be activated by PerC-like proteins from either organism. *J Bacteriol* **187:**458–472.

**78. Ahmer BM.** 2004. Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica*. *Mol Microbiol* **52:**933–945.

**79. Michael B, Smith JN, Swift S, Heffron F, Ahmer BM.** 2001. SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. *J Bacteriol* **183:**5733–5742.

**80. Yao Y, Martinez-Yamout MA, Dickerson TJ, Brogan AP, Wright PE, Dyson HJ.** 2006. Structure of the *Escherichia coli* quorum sensing protein SdiA: activation of the folding switch by acyl homoserine lactones. *J Mol Biol* **355:**262–273.

**81. Sharp FC, Sperandio V.** 2007. QseA directly activates transcription of LEE1 in enterohemorrhagic *Escherichia coli*. *Infect Immun* **75:**2432–2440.

**82. Sperandio V, Li CC, Kaper JB.** 2002. Quorum-sensing *Escherichia coli* regulator A: a regulator of the LysR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic *E. coli*. *Infect Immun* **70:**3085–3093.

**83. Reading NC, Rasko D, Torres AG, Sperandio V.** 2010. A transcriptome study of the QseEF two-component system and the QseG membrane protein in enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology* **156:**1167–1175.

**84. Rasko DA, Moreira CG, Li de R, Reading NC, Ritchie JM, Waldor MK, Williams N, Taussig R, Wei S, Roth M, Hughes DT, Huntley JF, Fina MW, Falck JR, Sperandio V.** 2008. Targeting QseC signaling and virulence for antibiotic development. *Science* **321:**1078–1080.

**85. Reading NC, Torres AG, Kendall MM, Hughes DT, Yamamoto K, Sperandio V.** 2007. A novel two-component signaling system that activates transcription of an enterohemorrhagic *Escherichia coli* effector involved in remodeling of host actin. *J Bacteriol* **189:**2468–2476.

**86. Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, Han CG, Ohtsubo E, Nakayama K, Murata T, Tanaka M, Tobe T, Iida T, Takami H, Honda T, Sasakawa C, Ogasawara N, Yasunaga T, Kuhara S, Shiba T, Hattori M, Shinagawa H.** 2001. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res* **8:**11–22.

**87. Perna NT, Plunkett G 3rd, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Posfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA, Blattner FR.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* **409:**529–533.

**88. Samadder P, Xicohtencatl-Cortes J, Saldana Z, Jordan D, Tarr PI, Kaper JB, Giron JA.** 2009. The *Escherichia coli* ycbQRST operon encodes fimbriae with laminin-binding and epithelial cell adherence properties in Shiga-toxigenic *E. coli* O157:H7. *Environ Microbiol* **11:**1815–1826.

**89. Doughty S, Sloan J, Bennett-Wood V, Robertson M, Robins-Browne RM, Hartland EL.** 2002. Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. *Infect Immun* **70:**6761–6769.

**90. Tatsuno I, Mundy R, Frankel G, Chong Y, Phillips AD, Torres AG, Kaper JB.** 2006. The *lpf* gene cluster for long polar fimbriae is not involved in adherence of enteropathogenic *Escherichia coli* or virulence of *Citrobacter rodentium*. *Infect Immun* **74:**265–272.

**91. Low AS, Holden N, Rosser T, Roe AJ, Constantinidou C, Hobman JL, Smith DG, Low JC, Gally DL.** 2006. Analysis of fimbrial gene clusters and their expression in enterohaemorrhagic *Escherichia coli* O157:H7. *Environ Microbiol* **8:**1033–1047.

**92. Xicohtencatl-Cortes J, Monteiro-Neto V, Saldana Z, Ledesma MA, Puente JL, Giron JA.** 2009. The type 4 pili of enterohemorrhagic *Escherichia coli* O157:H7 are multipurpose structures with pathogenic attributes. *J Bacteriol* **191:**411–421.

**93. Kim SH, Kim YH.** 2004. *Escherichia coli* O157:H7 adherence to HEp-2 cells is implicated with curli expression and outer membrane integrity. *J Vet Sci* **5:**119–124.

**94. Saldana Z, Xicohtencatl-Cortes J, Avelino F, Phillips AD, Kaper JB, Puente JL, Giron JA.** 2009. Synergistic role of curli and cellulose in cell adherence and biofilm formation of attaching and effacing *Escherichia coli* and identification of Fis as a negative regulator of curli. *Environ Microbiol* **11:**992–1006.

**95. Torres AG, Li Y, Tutt CB, Xin L, Eaves-Pyles T, Soong L.** 2006. Outer membrane protein A of *Escherichia coli* O157:H7 stimulates dendritic cell activation. *Infect Immun* **74:**2676–2685.

**96. Nicholls L, Grant TH, Robins-Browne RM.** 2000. Identification of a novel genetic locus that is required for in vitro adhesion of a clinical isolate of enterohaemorrhagic *Escherichia coli* to epithelial cells. *Mol Microbiol* **35:**275–288.

**97. Tarr PI, Bilge SS, Vary JCJ, Jelacic S, Habeeb RL, Ward TR, Baylor MR, Besser TE.** 2000. Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. *Infect Immun* **68:**1400–1407.

**98. Totsika M, Wells TJ, Beloin C, Valle J, Allsopp LP, King NP, Ghigo JM, Schembri MA.** 2012. Molecular characterization of the EhaG and UpaG trimeric autotransporter proteins from pathogenic *Escherichia coli*. *Appl Environ Microbiol* **78:**2179–2189.

**99. Grys TE, Siegel MB, Lathem WW, Welch RA.** 2005. The StcE protease contributes to intimate adherence of enterohemorrhagic *Escherichia coli* O157:H7 to host cells. *Infect Immun* **73:**1295–1303.

**100. Lathem WW, Grys TE, Witowski SE, Torres AG, Kaper JB, Tarr PI, Welch RA.** 2002. StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. *Mol Microbiol* **45:**277–288.

**101. Farfan MJ, Cantero L, Vidal R, Botkin DJ, Torres AG.** 2011. Long polar fimbriae of enterohemorrhagic *Escherichia coli* O157:H7 bind to extracellular matrix proteins. *Infect Immun* **79:**3744–3750.

**102. Torres AG, Milflores-Flores L, Garcia-Gallegos JG, Patel SD, Best A, La Ragione RM, Martinez-Laguna Y, Woodward MJ.** 2007. Environmental regulation and colonization attributes of the long polar fimbriae (LPF) of *Escherichia coli* O157:H7. *Int J Med Microbiol* **297:**177–185.

**103. Jordan DM, Cornick N, Torres AG, Dean-Nystrom EA, Kaper JB, Moon HW.** 2004. Long polar fimbriae contribute to colonization by *Escherichia coli* O157:H7 in vivo. *Infect Immun* **72:**6168–6171.

**104. Lloyd SJ, Ritchie JM, Rojas-Lopez M, Blumentritt CA, Popov VL, Greenwich JL, MWaldor MK, Torres AG.** 2012. A double, long polar fimbria mutant of *Escherichia coli* O157:H7 expresses Curli and exhibits reduced in vivo colonization. *Infect Immun* **80:**914–920.

**105. Torres AG, Lopez-Sanchez GN, Milflores-Flores L, Patel SD, Rojas-Lopez M, Martinez de la Pena CF, Arenas-Hernandez MM, Martinez-Laguna Y.** 2007. Ler and H-NS, regulators controlling expression of the long polar fimbriae of *Escherichia coli* O157:H7. *J Bacteriol* **189:**5916–5928.

**106. Rendon MA, Saldana Z, Erdem AL, Monteiro-Neto V, Vazquez A, Kaper JB, Puente JL, Giron JA.** 2007. Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. *Proc Natl Acad Sci USA* **104:**10637–10642.

**107. Martinez-Santos VI, Medrano-Lopez A, Saldana Z, Giron JA, Puente JL.** 2012. Transcriptional regulation of the *ecp* operon by EcpR, IHF, and H-NS in attaching and effacing *Escherichia coli*. *J Bacteriol* **194:**5020–5033.

**108. Lathem WW, Bergsbaken T, Welch RA.** 2004. Potentiation of C1 esterase inhibitor by StcE, a metalloprotease secreted by *Escherichia coli* O157:H7. *J Exp Med* **199:**1077–1087.

**109. Echtenkamp F, Deng W, Wickham ME, Vazquez A, Puente JL, Thanabalasuriar A, Gruenheid S, Finlay BB, Hardwidge PR.** 2008. Characterization of the NleF effector protein from attaching and effacing bacterial pathogens. *FEMS Microbiol Lett* **281:**98–107.

**110. Hemrajani C, Marches O, Wiles S, Girard F, Dennis A, Dziva F, Best A, Phillips AD, Berger CN, Mousnier A, Crepin VF, Kruidenier L, Woodward MJ, Stevens MP, La Ragione RM, MacDonald TT, Frankel G.** 2008. Role of NleH, a type III secreted effector from attaching and effacing pathogens, in colonization of the bovine, ovine, and murine gut. *Infect Immun* **76:**4804–4813.

**111. Kelly M, Hart E, Mundy R, Marches O, Wiles S, Badea L, Luck S, Tauschek M, Frankel G, Robins-Browne RM, Hartland EL.** 2006. Essential role of the type III secretion system effector NleB in colonization of mice by *Citrobacter rodentium*. *Infect Immun* **74:**2328–2337.

**112. Gruenheid S, Sekirov I, Thomas NA, Deng W, O'Donnell P, Goode D, Li Y, Frey EA, Brown NF, Metalnikov P, Pawson T, Ashman K, Finlay BB.** 2004. Identification and characterization of NleA, a non-LEE-encoded type III translocated virulence factor of enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol* **51:**1233–1249.

**113. Creuzburg K, Schmidt H.** 2007. Molecular characterization and distribution of genes encoding members of the type III effector nleA family among pathogenic *Escherichia coli* strains. *J Clin Microbiol* **45:**2498–2507.

**114. Mundy R, Jenkins C, Yu J, Smith H, Frankel G.** 2004. Distribution of espI among clinical enterohaemorrhagic and enteropathogenic *Escherichia coli* isolates. *J Med Microbiol* **53:**1145–1149.

**115. Roe AJ, Tysall L, Dransfield T, Wang D, Fraser-Pitt D, Mahajan A, Constandinou C, Inglis N, Downing A, Talbot R, Smith DG, Gally DL.** 2007. Analysis of the expression, regulation and export of NleA-E in *Escherichia coli* O157:H7. *Microbiology* **153:**1350–1360.

**116. Garcia-Angulo VA, Martinez-Santos VI, Villasenor T, Santana FJ, Huerta-Saquero A, Martinez LC, Jimenez R, Lara-Ochoa C, Tellez-Sosa J, Bustamante VH, Puente JL.** 2012. A distinct regulatory sequence is essential for the expression of a subset of *nle* genes in attaching and effacing *Escherichia coli*. *J Bacteriol* **194:**5589–5603.

**117. Schwidder M, Hensel M, Schmidt H.** 2011. Regulation of nleA in Shiga toxin-producing *Escherichia coli* O84:H4 strain 4795/97. *J Bacteriol* **193:**832–841.

**118. Njoroge J, Sperandio V.** 2012. Enterohemorrhagic *Escherichia coli* virulence regulation by two bacterial adrenergic kinases, QseC and QseE. *Infect Immun* **80:**688–703.

**119. Mellies JL, Haack KR, Galligan DC.** 2007. SOS regulation of the type III secretion system of enteropathogenic *Escherichia coli*. *J Bacteriol* **189:**2863–2872.

**120. Campellone KG, Robbins D, Leong JM.** 2004. EspFU is a translocated EHEC effector that interacts with Tir and N-WASP and promotes Nck-independent actin assembly. *Dev Cell* **7:**217–228.

**121. Garmendia J, Phillips AD, Carlier MF, Chong Y, Schuller S, Marches O, Dahan S, Oswald E, Shaw RK, Knutton S, Frankel G.** 2004. TccP is an enterohaemorrhagic *Escherichia coli* O157:H7 type III effector protein that couples Tir to the actin-cytoskeleton. *Cell Microbiol* **6:**1167–1183.

**122. Ritchie JM, Brady MJ, Riley KN, Ho TD, Campellone KG, Herman IM, Donohue-Rolfe A, Tzipori S, Waldor MK, Leong JM.** 2008. EspFU, a type III-translocated effector of actin assembly, fosters epithelial association and late-stage intestinal colonization by *E. coli* O157:H7. *Cell Microbiol* **10:**836–847.

**123. Sallee NA, Rivera GM, Dueber JE, Vasilescu D, Mullins RD, Mayer BJ, Lim WA.** 2008. The pathogen protein EspF(U) hijacks actin polymerization using mimicry and multivalency. *Nature* **454:**1005–1008.

**124. Garmendia J, Frankel G, Crepin VF.** 2005. Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: translocation, translocation, translocation. *Infect Immun* **73:**2573–2585.

**125. Njoroge JW, Gruber C, Sperandio V.** 2013. The interacting Cra and KdpE regulators are involved in the expression of multiple virulence factors in enterohemorrhagic *Escherichia coli*. *J Bacteriol* **195:**2499–2508.

**126. Tyler JS, Mills MJ, Friedman DI.** 2004. The operator and early promoter region of the Shiga toxin type 2-encoding bacteriophage 933W and control of toxin expression. *J Bacteriol* **186:**7670–7679.

**127. O'Brien AD, LaVeck GD, Thompson MR, Formal SB.** 1982. Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. *J Infect Dis* **146:**763–769.

**128. Wagner PL, Livny J, Neely MN, Acheson DW, Friedman DI, Waldor MK.** 2002. Bacteriophage control of Shiga toxin 1 production and release by *Escherichia coli*. *Mol Microbiol* **44:**957–970.

**129. Waldor MK, Friedman DI.** 2005. Phage regulatory circuits and virulence gene expression. *Curr Opin Microbiol* **8:**459–465.

**130. Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, Acheson DW.** 2000. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J Infect Dis* **181:**664–670.

**131. Los JM, Los M, Wegrzyn A, Wegrzyn G.** 2010. Hydrogen peroxide-mediated induction of the Shiga toxin-converting lambdoid prophage ST2-8624 in *Escherichia coli* O157:H7. *FEMS Immunol Med Microbiol* **58:**322–329.

**132. Vareille M, de Sablet T, Hindre T, Martin C, Gobert AP.** 2007. Nitric oxide inhibits Shiga-toxin synthesis by enterohemorrhagic *Escherichia coli*. *Proc Natl Acad Sci USA* **104:**10199–10204.

**133. Reading NC, Rasko DA, Torres AG, Sperandio V.** 2009. The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. *Proc Natl Acad Sci USA* **106:**5889–5894.

**134. Ogura Y, Ooka T, Iguchi A, Toh H, Asadulghani M, Oshima K, Kodama T, Abe H, Nakayama K, Kurokawa K, Tobe T, Hattori M, Hayashi T.** 2009. Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. *Proc Natl AcadSci USA* **106:**17939–17944.

**135. Li H, Granat A, Stewart V, Gillespie JR.** 2008. RpoS, H-NS, and DsrA influence EHEC hemolysin operon (ehxCABD) transcription in *Escherichia coli* O157:H7 strain EDL933. *FEMS Microbiol Lett* **285:**257–262.

**136. Gottesman S.** 2004. The small RNA regulators of *Escherichia coli*: roles and mechanisms. *Annu Rev Microbiol* **58:**303–328.

**137. Saitoh T, Iyoda S, Yamamoto S, Lu Y, Shimuta K, Ohnishi M, Terajima J, Watanabe H.** 2008. Transcription of the *ehx* enterohemolysin gene is positively regulated by GrlA, a global regulator encoded within the locus of enterocyte effacement in enterohemorrhagic *Escherichia coli*. *J Bacteriol* **190:**4822–4830.

**138. House B, Kus JV, Prayitno N, Mair R, Que L, Chingcuanco F, Gannon V, Cvitkovitch DG, Barnett Foster D.** 2009. Acid-stress-induced changes in enterohaemorrhagic *Escherichia coli* O157:H7 virulence. *Microbiology* **155:**2907–2918.

**139. Calderon VE, Chang Q, McDermott M, Lytle MB, McKee G, Rodriguez K, Rasko DA, Sperandio V, Torres AG.** 2010. Outbreak caused by cad-negative Shiga toxin-producing *Escherichia coli* O111, Oklahoma. *Foodborne Pathog Dis* **7:**107–109.

**140. Fabich AJ, Jones SA, Chowdhury FZ, Cernosek A, Anderson A, Smalley D, McHargue JW, Hightower GA, Smith JT, Autieri SM, Leatham MP, Lins JJ, Allen RL, Laux DC, Cohen PS, Conway T.** 2008. Comparison of carbon nutrition for pathogenic and commensal *Escherichia coli* strains in the mouse intestine. *Infect Immun* **76:**1143–1152.

**141. Kamada N, Kim Y-G, Sham HP, Vallance BA, Puente JL, Martens EC, Núñez G.** 2012. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. *Science* **336:**1325–1329.

**142. Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, Koga Y, Sudo N.** 2012. Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. *Am J Physiol Gastrointest Liver Physiol* **303:**G1288–G1295.

**143. Hersh BM, Farooq FT, Barstad DN, Blankenhorn DL, Slonczewski JL.** 1996. A glutamate-dependent acid resistance gene in *Escherichia coli*. *J Bacteriol* **178:**3978–3981.

**144. McGannon CM, Fuller CA, Weiss AA.** 2010. Different classes of antibiotics differentially influence Shiga toxin production. *Antimicrob Agents Chemother* **54:**3790–3798.

**145. Wagner PL, Neely MN, Zhang X, Acheson DW, Waldor MK, Friedman DI.** 2001. Role for a phage promoter in Shiga toxin 2 expression from a pathogenic *Escherichia coli* strain. *J Bacteriol* **183:**2081–2085.

**146. Torres AG, Slater TM, Patel SD, Popov VL, Arenas-Hernandez MM.** 2008. Contribution of the Ler- and H-NS-regulated long polar fimbriae of *Escherichia coli* O157:H7 during binding to tissue-cultured cells. *Infect Immun* **76:**5062–5071.

**147. Carter MQ, Parker CT, Louie JW, Huynh S, Fagerquist CK, Mandrell RE.** 2012. RcsB contributes to the distinct stress fitness among *Escherichia coli* O157:H7 curli variants of the 1993 hamburger-associated outbreak strains. *Appl Environ Microbiol* **78:**7706–7719.

**148. Lim J, Lee KM, Kim SH, Kim Y, Kim SH, Park W, Park S.** 2011. YkgM and ZinT proteins are required for maintaining intracellular zinc concentration and producing curli in enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 under zinc deficient conditions. *Int J Food Microbiol* **149:**159–170.

**149. Sharma VK, Bearson BL.** 2013. Hha controls *Escherichia coli* O157:H7 biofilm formation by differential regulation of global transcriptional regulators FlhDC and CsgD. *Appl Environ Microbiol* **79:**2384–2396.

**150. Baev MV, Baev D, Radek AJ, Campbell JW.** 2006. Growth of *Escherichia coli* MG1655 on LB medium: monitoring utilization of amino acids, peptides, and nucleotides with transcriptional microarrays. *Appl Microbiol Biotechnol* **71:**317–322.

**151. Douchin V, Bohn C, Bouloc P.** 2006. Down-regulation of porins by a small RNA bypasses the essentiality of the regulated intramembrane

proteolysis protease RseP in *Escherichia coli*. *J Biol Chem* **281:**12253–12259.

**152. Udekwu KI, Darfeuille F, Vogel J, Reimegard J, Holmqvist E, Wagner EG.** 2005. Hfq-dependent regulation of OmpA synthesis is mediated by an antisense RNA. *Genes Dev* **19:**2355–2366.

**153. Stevens MP, Roe AJ, Vlisidou I, van Diemen PM, La Ragione RM, Best A, Woodward MJ, Gally DL, Wallis TS.** 2004. Mutation of toxB and a truncated version of the efa-1 gene in *Escherichia coli* O157:H7 influences the expression and secretion of locus of enterocyte effacement-encoded proteins but not intestinal colonization in calves or sheep. *Infect Immun* **72:**5402–5411.

**154. Rashid RA, Tabata TA, Oatley MJ, Besser TE, Tarr PI, Moseley SL.** 2006. Expression of putative virulence factors of *Escherichia coli* O157:H7 differs in bovine and human infections. *Infect Immun* **74:**4142–4148.

**155. Rashid RA, Tarr PI, Moseley SL.** 2006. Expression of the *Escherichia coli* IrgA homolog adhesin is regulated by the ferric uptake regulation protein. *Microb Pathog* **41:**207–217.

## Author Queries

**Q1:** Please confirm Emily Lorenzen is also at the same institution in the affiliation line.
**Q2:** Is this the correct definition of LRP?