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Nutrient and chemical sensing by intestinal pathogens

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Abstract

Pathogenic gut bacteria, such as those comprising the Enterobacteriaceae family, have evolved sophisticated virulence mechanisms, including nutrient and chemical sensing, to escape host defense strategies and produce disease. In this review we describe the mechanisms utilized by the enteric pathogen enterohemorrhagic *E. coli* (EHEC) O157:H7 to achieve successful colonization of its mammalian host.

Keywords

Enterobacteriaceae; Signals; Type-III secretion; Two-component system

1. Introduction

The gastrointestinal tract plays home to trillions of bacteria that perform essential functions for the host. Pathogenic bacteria must compete with the commensal bacteria in order to thrive and cause disease [1–4]. Successful colonization of the human gut requires that pathogens be skillful users of virtually any molecule available in the gut. The human gut contains two zones with different nutrient/chemical sources for bacteria: the lumen with stool deposits and an epithelial layer rich in mucins. Human stool is rich in “waste” materials originating from the diet and include polysaccharides (starch, hemicellulose), hormones (cortisol, serotonin), secondary metabolites from microflora fermentation (acetate, butyrate, indole-3-propionate), salts and minerals [5–6]. The colonic mucosal layer is further divided into two layers: a loose outer layer that can be utilized by the gut microflora and a tight inner layer free of bacteria [7–8]. The outer layer is composed primarily of *O*-glycan proteins that can serve as nutrients and potential attachment sites [9].

The diet consumed by the host greatly influences which nutrients are available within the gut, in turn affecting the composition of bacteria within the intestine. Two major phyla dominate the adult gut: the Bacteroidetes and the Firmicutes phyla. The Bacteroidetes phyla contain *Bacteroides* and *Prevotella* species while the Firmicutes contains *Lactobacillus* and *Clostridium* species [10]. Compelling evidence indicates the correlation between human diseases such as obesity and shifts in bacterial populations [11]. An obesity mouse model indicates a strong correlation between higher consumption of a Westernized diet rich in

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saturated fats and complex oligosaccharides and a shift towards a large number of Firmicutes [12]. A study comparing the composition of the gut microflora between individuals consuming a Westernized diet and individuals ingesting a vegetarian diet indicated that consumption of a Westernized diet increased the *Bacteroides* species in the gut whereas consumption of a vegetarian diet increased the *Prevotella* species [13].

The majority of human gut pathogens belong to the Gamma-Proteobacteria class, including the intestinal pathogen that will be covered in this review: Enterohemorrhagic *E. coli* (EHEC) O157:H7. This pathogen is a Gram negative bacterium that employs a syringe-like type III secretion system (T3SS) to assist in its colonization of the gut by injection of effectors into human epithelial cells that change host signaling pathways [14]. Bacteria sense chemicals and nutrients in their environment and engage many transcriptional regulators, including two-component signalling systems (TCS) to regulate their gene expression [15]. A TCS consists of a histidine kinase (HK) sensor protein that autophosphorylates in response to environmental cues and transfers this phosphate to a response regulator (RR) protein, which is generally a transcription factor. TCSs allow bacteria to control cellular functions and respond to environmental conditions including pH, nutrient availability and metabolic end products [15].

2. Enterohemorrhagic *E. coli* (EHEC) O157:H7

Earlier studies involving pathogenic *E. coli* were designed to determine the role of quorum sensing (QS) in the expression of virulence factors in enterohemorrhagic (EHEC) and enteropathogenic *E. coli* (EPEC) [16]. *E. coli* uses several QS systems, such as the *luxS*/autoinducer-2 (AI-2) [16–17], Autoinducer-3 (AI-3)/epinephrine/norepinephrine [18–19], indole [20], and the LuxR homolog SdiA [21–22] to achieve intercellular signaling. The majority of these signaling systems are involved in interspecies communication, and the AI-3/epinephrine/norepinephrine signaling system is also involved in inter-kingdom communication [19]. EPEC is responsible for causing watery diarrhea in children, while EHEC causes bloody diarrhea and the life-threatening hemolytic-uremic syndrome (HUS). EHEC produces Shiga toxin while EPEC does not. However, both types can cause intestinal lesions known as attaching and effacing (AE) lesions [23]. A pathogenicity island (PAI) called the locus of enterocyte effacement (LEE) encodes a cluster of genes, including those responsible for AE lesions [24]. The LEE encode a T3SS [25], an adhesin (intimin) [26] and its receptor (Tir) [27], and effector proteins [28–32]. The *ler* gene encodes for the master regulator of the LEE genes [23, 33–35]. An initial study [16] demonstrated that expression of the LEE in EPEC and EHEC O157:H7 is regulated by QS. In addition to the LEE, flagellar expression and motility, and Shiga toxin expression can also be controlled by QS [36–37]. Initial findings suggested that the bacterial-derived signal AI-2 was essential for LEE expression [16]; however, addition of purified and synthesized AI-2 to *in vitro* cultures did not restore LEE expression in an EHEC O157:H7 *luxS* mutant [38–39]. *LuxS* catalyzes the final reaction of ribosyl-homocysteine into 4,5-dihydroxy-2,3-pentanedione (DPD) for the synthesis of AI-2 [40–41]; thus, it was hypothesized that another autoinducer molecule must be responsible for controlling virulence factors in EHEC [19]. Interruption of *luxS* affects metabolism and reduces production of another chemical signal, AI-3 [42]. A different QS signal, AI-3 overcame the mutation in *luxS*, activated the LEE, and restored motility.

Many commensal and pathogenic bacteria from the gut produce both AI-2 and AI-3 [19]. One hypothesis is that the AI-3 system might be used by pathogenic strains, like EHEC O157:H7, to alert the bacterium of its arrival to the large intestine and initiate virulence gene expression [16, 19]. Clarke et al., [43] demonstrated that AI-3, as well as the host hormones epinephrine and norepinephrine, signal through the TCS QseBC, with QseC directly sensing

these three signals, to activate flagella expression. From these findings, it was proposed that bacteria and the host communicate with one another using inter-kingdom signalling [43].

2.1 The autoinducer-3/epinephrine/norepinephrine system coordination of flagella-motility genes using the two component system QseBC in *E. coli*

The QseBC TCS is composed of the membrane-spanning HK kinase, QseC, and the RR QseB. The QseC sensor kinase contains two domains: a histidine kinase domain and an ATPase domain. QseC senses the signals AI-3, epinephrine, and norepinephrine to coordinate virulence gene expression, including expression of flagella and motility through the flagella master regulator FlhDC [44]. Activation of *flhDC* depends on phosphorylated QseB. The phosphorylated QseB protein binds to two different regions of the *flhDC* promoter: the proximal region (−300bp to +50bp) and a distal region (−900bp to −650bp). The phospho-QseB binds first to the distal region, then later to the proximal region [45–46]. Transcription of *flhDC* occurs in response to a coordinated process dependent on the intensity of the signal received by QseC. If there is a low input signal, then QseB will not be phosphorylated and the protein will bind to a region between −650 and −300 bp which may result in flagella repression [45–46]. At high levels of input signal, a phosphorylated QseB will bind to both the distal and proximal regions of the *flhDC* operon and flagella is activated [45–46].

2.2 O157:H7 controls LEE expression by employing several chemical and nutrient sensing mechanisms

2.2.1. Cra and KdpE regulation of the LEE

The LEE is arranged into five major operons *LEE1* to *LEE5* and encodes a T3SS that permits the attachment and delivery of effectors proteins into the host cell [24–25]. Bacterial effectors induce rearrangement and accumulation of host actin to form the hallmark AE lesions that cup the bacterium tightly to the intestinal epithelium reviewed by Garmendia et al., [47]. Regulation of LEE expression is primarily led by the locus of enterocyte regulator (Ler) [23, 33–35]. Transcriptional activation of *ler* is complex and also includes several TCS that interconnect with each other [33]. QseC does not activate exclusively its cognate RR QseB, but also interacts with other RRs such as KdpE and QseF. KdpE is a transcriptional regulator that together with KdpD, its cognate sensor kinase, is responsible for sensing potassium [48]. Meanwhile, QseF is a RR that forms a TCS with the QseE histidine sensor kinase (HK), which senses the host hormone epinephrine, sulfate and phosphate sources [49]. QseC transfers a phosphate to both KdpE and QseF, which in turn activate expression of the LEE and Shiga toxin, respectively [46] (Fig. 1).

In addition to the marked effect on LEE expression by AI-3 and epinephrine, other environmental cues such as sugars affect LEE transcription. Animal studies indicated that EHEC uses glycolytic substrates for initial colonization and only displayed a preference for gluconeogenic carbon sources in the context of competition with commensal *E. coli* [50]. Investigations conducted *in vitro* have identified a transcriptional regulator protein named catabolite repressor activator (Cra), which is a global regulator for carbon metabolism [51]. Cra senses sugar concentrations to regulate its targeted genes. Another transcriptional regulator involved with homeostatic maintenance in *E. coli* and the regulation of the LEE in EHEC is KdpE [46]. Cra and KdpE regulate expression of the LEE in accordance to the amount of glucose available [52]. If the medium is rich in glucose (glycolysis), EHEC will accumulate fructose-1,6-phosphate (FBP) and reduce Cra binding to the *ler* promoter, thus inhibiting LEE expression. However, in limited glucose environments (gluconeogenesis), succinate production will decrease the availability of FBP that promotes Cra binding and increases LEE expression [52]. Similar to Cra, KdpE enhances *ler* transcription only under gluconeogenic conditions and reduces its binding capacity to the *ler* promoter region during

glycolysis. Under conditions of high glucose availability (glycolytic), IIA^{Ntr} is dephosphorylated, and only in its dephosphorylated form binds to the KdpD HK (the cognate HK for KdpE) increasing its activity, and consequently KdpE phosphorylation, leading to higher expression of the KdpE target genes *kdpFABC* [53]. However, KdpE preferentially binds to the *ler* promoter in the dephosphorylated form, which is more abundant under gluconeogenic conditions [54]. Both proteins interact with each other to optimally activate the LEE, indicating a strategy for EHEC to regulate gene expression during colonization where the environment is more gluconeogenic [52] (Fig. 1). EHEC competes with commensal *E. coli* (the predominant species within the *-Proteobacteria*) for the same carbon sources during growth within the mammalian intestine [3, 55–58]. EHEC uses glycolytic substrates for initial growth, but is unable to effectively compete for these carbon sources beyond the first few days, and begins to utilize gluconeogenic substrates to stay within the intestine [55]. Hence, it is advantageous to coordinate expression of the LEE with these environmental conditions. Commensal *E. coli* can be found in the lumen and outer mucus layer, which is glycolytic due to the abundant sugar sources supplied by the glycolytic microbiota, while the interface with the epithelium is a more gluconeogenic environment. Hence the KdpE/Cra-dependent activation of the LEE under gluconeogenic conditions ensures that these genes only be optimally expressed at the epithelium interface, and not in the lumen.

2.2.2. Fucose regulation of LEE expression—In addition to Cra and KdpE sugar dependent regulation of the LEE, there is a newly identified EHEC TCS that is repressed by the QseC and QseE signaling systems. This TCS, named FusKR, represses expression of the LEE genes [59]. The genes encoding *fusKR* are clustered in a PAI (OI-20) only present in EPEC O55:H7 (the *E. coli* lineage that gave rise to EHEC O157:H7) [60–61], EHEC O157:H7 and *C. rodentium*, AE gastrointestinal pathogens that colonize the colon. Horizontal acquisition of PAIs contributes to virulence of an organism, allowing exploitation of other niches and hosts for colonization [62]. The interplay between ancient and recent evolutionary acquisitions has shaped EHEC pathogenicity. Interestingly, EHEC's ancestor, EPEC O55:H7 [61], is the only other serotype of *E. coli* to harbor *fusKR*, suggesting that acquisition of these genes is recent. The recent acquisition of OI-20 on EHEC evolution provided this pathogen with a novel signal transduction system. OI-20 genes are up-regulated when EHEC is grown in the presence of mucus [63], and during infection of the colonic mucus-producing cell line HT29 [59], suggesting that expression of this TCS in mucus facilitates EHEC adaptation to the mammalian intestine. Thus, it is tempting to speculate that acquisition of OI-20 enhances EHEC's capability to successfully compete for a niche in the colon. FusR encodes a RR that directly represses expression of the LEE genes, by repressing transcription of *ler* (Fig. 1). FusK, the sensor component of this TCS, autophosphorylates in response to fucose, thus revealing for the first time a signal transduction mechanism that senses fucose to regulate expression of the LEE, as well as EHEC intestinal colonization in the infant rabbit model of infection [59]. In addition to LEE regulation, FusKR also indirectly represses expression of the *fuc* genes involved in fucose utilization through regulation of the Z0461 hexose-phosphate-major facilitator-superfamily (MFS) transporter, also encoded within the OI-20. Consequently the *fusK* and *fusR* mutants grow faster in fucose as a sole carbon source than WT EHEC, and this response is specific to fucose, with the mutants and WT growing at similar rates with other C-sources (galactose, glucose, rhamnose and xylose) [59].

Fucose is one of the major components of mucin glycoproteins, and it is highly abundant in the intestine [64–66]. Primary fermenters such as *Bacteroides* are the gateway for the entrance of carbohydrates in the network of syntrophic links in the microbiota [67]. *Bacteroides thetaiotaomicron* (*B. theta*) produces multiple fucosidases that can cleave fucose from host glycans, resulting in high fucose availability in the gut lumen [66–69]. During

growth in mucin, *B. theta* contributes to *ler* regulation by cleaving fucose from mucin, thereby activating the FusKR signaling cascade that leads to repression of *ler*. In aggregate, these findings suggest that EHEC uses fucose, a host-derived signal made available by the microbiota, to modulate EHEC pathogenicity. FusKR repression of LEE expression in the mucus-layer prevents superfluous energy expenditure. Once in close contact to the epithelial surface, the QseCE adrenergic sensing-systems are triggered to activate virulence both directly through the QseCE cascade, and indirectly by repression of *fusKR*. EHEC competes with commensal *E. coli* (α -Proteobacteria), but not *B. theta*, for the same C-sources (e.g. fucose) within the mammalian intestine [3, 55–58, 70]. Commensal *E. coli*, however, are not found in close contact with the epithelia, being in the mucus-layer, where it is counter-productive for EHEC to invest resources to utilize fucose, when EHEC can efficiently use other C-sources such as: galactose, hexorunates, and mannose, which are not used by commensal *E. coli* within the intestine [57]. Additionally, in contrast to commensal *E. coli*, EHEC is found closely associated with the intestinal epithelium [55]. Therefore, EHEC can utilize nutrients exclusively available at the surface of the epithelial cells. Consequently, the decreased expression of the fuc operon through fucose-sensing by FusKR, may prevent EHEC from expending energy in fucose utilization in the mucus-layer, where it competes with commensal *E. coli* for this resource, and focus on utilizing other C-sources (e.g. galactose, whose utilization is not affected by FusKR [59]), not used by this competitor. Thus, the colonization defect of *fusK* of the mammalian GI tract results from its inability to correctly time virulence and metabolic gene expression [59].

Linking metabolism to the precise coordination of virulence expression is a key step in the adaptation of pathogens towards niche recognition of suitable sites for colonization. Indeed the *fusK* mutant is attenuated for mammalian infection [59]. It is known that the invading enteric pathogen *C. rodentium* (a murine pathogen that models the enteric infection of the human pathogen EHEC, and also harbors Cra, KdpE and FusKR) causes inflammation within the gut that in turn diminishes the overall numbers of bacteria in the microbiota, acting as an initial competitive advantage to the pathogen [71–72]. Additionally, infection with *C. rodentium* also causes significant changes in the structure of the microbial community, decreasing the number of anaerobes (such as *Bacteroidetes*), and increasing the numbers of α -Proteobacteria [71]. α -Proteobacteria normally constitute a minute portion of the microbiota in healthy individuals, but this scenario quickly changes in pathogen-induced dysbiosis, where there is a marked increase in the prevalence of α -Proteobacteria [73]. This has important consequences towards niche competition. *C. rodentium* can be outcompeted by other α -Proteobacteria such as *E. coli*, but not by *Bacteroides*, and this competition is governed by carbon source availability. *Bacteroides* can utilize complex polysaccharides as carbon sources, while α -Proteobacteria (*E. coli* and *C. rodentium*) are restricted to monosaccharide utilization. The shift on the microbial composition towards the nutrient competing α -Proteobacteria sets up *C. rodentium* for failure regarding long term host colonization [3]. The link of carbon metabolism and virulence expression is a key step in the adaptation of pathogens towards recognition of suitable sites for colonization and contributes to the dynamic and volatile interactions between the host, pathogens and the microbiota.

3. Conclusions

There is an intricate relationship between nutrient sources in microbiota and pathogen relationships. Glycophagic members of the microbiota, such as *B. theta*, make fucose from mucin accessible to EHEC, and EHEC interprets this information to recognize that it is in the lumen, where expression of its LEE-encoded T3SS is onerous and not advantageous. Using yet another nutrient-based environmental cue, EHEC also times LEE expression through recognition of glycolytic and gluconeogenic environments. The lumen is more

glycolytic due to predominant glycolytic members of the microbiota degrading complex polysaccharides into monosaccharides that can be readily utilized by non-glycolytic bacterial species such as *E. coli* and *C. rodentium* [3, 55–58]. In contrast, the tight mucus layer between the lumen and the epithelial interface in the GI tract is devoid of microbiota, it is known as a “zone of clearance” [8]. At the epithelial interface the environment is highly regarded as gluconeogenic [3, 55–58]. Hence, the coupling of LEE regulation to optimal expression under gluconeogenic and low fucose conditions, mirrors the interface with the epithelial layer environment in the GI tract, ensuring that EHEC will only express the LEE at optimal levels to promote AE lesion formation at the epithelial interface

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Fig.1.

Schematic depiction of the regulation of virulence factors in EHEC. Environmental signals present in the gut (autoinducer-3 [AI-3], epinephrine [Epi], norepinephrine [NE], SO₄, L-Fucose, Glucose) can trigger a series of signaling cascades to regulate virulence gene expression. QseC: quorum sensing *E. coli* regulator C. QseB: quorum sensing *E. coli* regulator B. QseE: quorum sensing *E. coli* regulator E. QseF: quorum sensing *E. coli* regulator F. FusK: Fucose sensing histidine kinase. FusR: Fucose sensing response regulator. LEE: locus of enterocyte effacement. Ler: LEE encoded regulator.