MINIREVIEW

Quorum sensing: the many languages of bacteria
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Abstract

In the conventional view of prokaryotic existence, bacteria live unicellularly, with responses to external stimuli limited to the detection of chemical and physical signals of environmental origin. This view of bacteriology is now recognized to be overly simplistic, because bacteria communicate with each other through small ‘hormone-like’ organic compounds referred to as autoinducers. These bacterial cell-to-cell signaling systems were initially described as mechanisms through which bacteria regulate gene expression via cell density and, therefore, they have been collectively termed quorum sensing. The functions controlled by quorum sensing are varied and reflect the needs of a particular species of bacteria to inhabit a given niche. Three major quorum-sensing circuits have been described: one used primarily by Gram-negative bacteria, one used primarily by Gram-positive bacteria, and one that has been proposed to be universal.

Introduction

Quorum sensing (QS) is a cell-to-cell signaling mechanism that refers to the ability of bacteria to respond to chemical hormone-like molecules called autoinducers. When an autoinducer reaches a critical threshold, the bacteria detect and respond to this signal by altering their gene expression. QS was first described in the regulation of bioluminescence in Vibrio fischeri and Vibrio harveyi (Nealson et al., 1970; Nealson & Hastings, 1979), and since then shown to be a widespread mechanism of gene regulation in bacteria. In this review, we will explore several QS systems used by bacteria; the LuxR/I-type systems, primarily used by Gram-negative bacteria, in which the signaling molecule is an acyl-homoserine lactone (AHL), the peptide signaling systems used primarily by Gram-positive bacteria, the luxS/AI-2 signaling used for interspecies communication, and the AI-3/epinephrine/norepinephrine interkingdom signaling system.

The LuxR/I signaling system

The LuxR/I system was the first one to be described in V. fischeri (Nealson et al., 1970). The luciferase operon in V. fischeri is regulated by two proteins, LuxI, which is responsible for the production of the AHL autoinducer, and LuxR, which is activated by this autoinducer to increase transcription of the luciferase operon (Engelbrecht et al., 1983; Engelbrecht & Silverman, 1984). Since this initial description, homologs of LuxR–LuxI have been identified in other bacteria, and in all of these LuxR–LuxI systems, the bacteria produce an AHL autoinducer, which binds to the LuxR protein and regulates the transcription of several genes involved in a variety of phenotypes. These include the production of antibiotics in Erwinia, motility in Yersinia pseudotuberculosis, and pathogenesis and biofilm formation in Pseudomonas aeruginosa, among others (Davies et al., 1998; de Kievit & Iglewski, 2000; Parsek & Greenberg, 2000) (Fig. 1).

The LuxI-type proteins are the AHL synthases. AHLs have a conserved homoserine lactone ring connected through an amide bond to a variable acyl chain. Acyl chains vary in number of carbons from four to 18 and the third position may or may not be modified (carbonyl group, hydroxyl or fully reduced). Different acyl chains ensure that different AHLs will be recognized by different LuxR-type proteins. The substrate used by LuxI-type proteins for AHL synthesis is S-adenosyl-methionine (SAM) to synthesize the homoserine lactone ring, and the acyl chains come from lipid metabolism, carried by various acyl-carrier proteins.
caused abnormal cell division, reduced adherence to cul-
the cell division genes
not produce AHLs (Swift number plasmid in enterohemorrhagic 
(2000) found that expression of SdiA from a high copy
The 
1999, 2001; Zhang, 2002). The LuxR-type proteins usually recog-
nize a specific AHL. Because of this feature, this
signal, they are targeted to degradation (Zhu & Winans,
1999).)
The LuxR-type proteins are transcription factors, which,
upon binding to the AHL signal, regulate transcription of
their target genes. It has been shown that AHL binding to
these proteins stabilizes them; otherwise, in the absence
of signal, they are targeted to degradation (Zhu & Winans,
1999, 2001; Zhang, 2002). The LuxR-type proteins usually
recognize a specific AHL. Because of this feature, this
signaling system has been primarily associated with intras-
pecies signaling. However, there are examples of LuxR-type
proteins (such as SdiA described below) that recognize more
than one AHL and are primarily involved in interspecies
signaling.

Escherichia coli and Salmonella have a LuxR homolog,
SdiA (Wang et al., 1991), but do not have a luxI gene, and
do not produce AHLs (Swift et al., 1999; Michael et al.,
2001). The E. coli sdiA gene initially was isolated as a regulator of
the cell division genes ftsQAZ (Wang et al., 1991). Although
a cloned sdiA gene on a multicopy plasmid can upregulate expression of ftsQAZ genes, an sdiA mutant has no apparent
cell division defects (Wang et al., 1991). Kanamaru et al.
(2000) found that expression of SdiA from a high copy number
plasmid in enterohemorrhagic E. coli (EHEC) caused abnormal cell division, reduced adherence to cul-
tured epithelial cells, and reduced expression of the intimin
adhesin protein and the EspD protein, both of which are
encoded on the locus of enterocyte effacement (LEE)
pathogenicity island. However, no sdiA EHEC mutant was
constructed and tested, and, consequently, the effects seen
could be artifacts because of the abnormally high expression of SdiA. Because no E. coli genes from either EHEC or K-12
have yet been demonstrated to be regulated by the single
chromosomal copy of sdiA, Ahmer (2004) recently con-
cluded that there are no confirmed members of a SdiA
regulon in this species. The precise role of SdiA in QS was
elusive for several years until Michael et al. (2001) reported
that SdiA is not sensing an autoinducer produced by
Salmonella itself, but rather AHLs produced by other
bacterial species. SdiA regulates a few genes in Salmonella
including one gene potentially involved in resistance to
human complement, rck (Ahmer et al., 1998). However,
mutation of the sdiA gene had no effect on virulence of
Salmonella in mouse, chicken or bovine models of disease
(Ahmer, 2004).

One of the best characterized LuxR/I-type QS systems is
in P. aeruginosa. Pseudomonas aeruginosa uses QS to activate
several genes involved in colonization and persistence within
the host (Parsek & Greenberg, 2000). Pseudomonas aerugi-

lessness as an opportunist pathogen of immunocompromised
individuals, including those with burns, human immuno-
deficiency virus, or cystic fibrosis (Parsek & Greenberg,
2000). The morbidity and mortality associated with cystic
fibrosis, in particular, are because of the chronic coloniza-
tion of the pulmonary airways by P. aeruginosa. QS controls
production of an array of virulence factors (elastase, exotox-
in A, piocinain, etc.) and biofilm development in this
organism. Disruption of the QS system diminishes P.
aeruginosa virulence in plants and animals and inhibits
biofilm formation (Rahme et al., 1995; Costerton et al.,
1999; Tan et al., 1999). The QS system of P. aeruginosa is very
complex and hierarchical. Pseudomonas aeruginosa produces
two AHLs, N-(3-oxododecanoyl)-l-homoserine lactone
(3OC12-HSL) and N-butanoyl-l-homoserine lactone (C4-
HSL) (Pearson et al., 1994, 1995). These AHLs bind to and
activate LasR and RhlR transcription factors, respectively
(Parsek & Greenberg, 2000). LasR complexed with 3OC12-
HSL activates the transcription of rhlR and rhlI (Rhl is the
synthase for C4-HSL). Therefore, LasR is at the very top of
the P. aeruginosa QS signaling cascade (Parsek & Greenberg,
2000).

Some bacteria have the ability to disrupt QS signaling by
degrading AHL autoinducers. The soil bacterium Bacillus
produces a lactonase enzyme that hydrolyzes the lactone
ring of AHLs. This lactonase enzyme probably interferes
with AHL signaling by other bacterial species with which
Bacillus competes in nature (Dong et al., 2001). In addition,
transgenic plants expressing the Bacillus lactonase show
resistance to QS-dependent bacterial infection (Dong et al., 2001).

The LuxR/I-type QS systems have been linked to interkingdom signaling. The marine macroalga Delisea pulchra blocks interaction of the plant pathogen Serratia liquifaciens by producing a halogenated furanone that acts as a competitive inhibitor of the bacterium's AHL-based QS system (Rasmussen, 2000). Another example of crosskingdom inhibitive inhibitor of the bacterium's AHL-based QS system by producing a halogenated furanone that acts as a competitive inhibitor of the plant pathogen Serratia liquifaciens Delisea pulchra. The marine macroalga Delisea pulchra blocks interaction of the plant pathogen Serratia liquifaciens by producing a halogenated furanone that acts as a competitive inhibitor of the bacterium's AHL-based QS system (Rasmussen, 2000). Another example of crosskingdom signaling is the marine macroalga Delisea pulchra, which produces a halogenated furanone that acts as a competitive inhibitor of the bacterium's AHL-based QS system (Rasmussen, 2000).

In addition, exposure of plants to AHLs induced the secretion of compounds that mimic QS signals and thus have the potential to disrupt QS in associated bacteria. There are also a growing number of reports indicating that bacterial AHLs modulate gene expression of mammalian organisms. Most of these studies implicate the 3OC12-HSL of P. aeruginosa as influencing the production of several cytokines by immune cells ‘in vitro’ and ‘in vivo’ (Telford, 1998; Smith et al., 2001, 2002a, b; Ritchie et al., 2003; Tateda, 2003). However, some of these reports appear to be contradictory with respect to whether this crosskingdom signaling is beneficial or detrimental to the host. Telford (1998) reported that 3OC12-HSL inhibited the production of interleukin-12 (IL-12) and tumor necrosis factor α by lipopolysaccharide-stimulated macrophages. Using an ‘in vitro’ model of B-cell activation, these authors also reported that production of immunoglobulin G1 antibodies and IgE was elevated by administration of 3OC12-HSL, leading this group to propose that this AHL acts to modulate a T-cell-mediated immune response from a type 1 (Th1, proinflammatory) response to a type 2 (Th2, anti-inflammatory) response. In contrast, this autoinducer has also been shown to increase the production of cyclo-oxygenase-2 and prostaglandin E2 in human lung fibroblasts (Smith et al., 2002a, b) and several proinflammatory chemokines, including IL-8, in human bronchial epithelial cells and lung fibroblasts (Smith et al., 2001). These results led Smith et al. (2001) to propose that the severe lung damage that accompanies P. aeruginosa infections is caused by an exuberant neutrophil response stimulated by AHL-induced IL-8. These conflicting data may be the result of different host cell types or concentrations of AHLs used. Mammalian cell interference could also offer an explanation to the contradictory results reported concerning 3OC12-HSL modulation of immune responses. Chun et al. (2004) reported that human airway epithelia inactivated 3OC12-HSL. They also reported that the AHL inactivation capability varied widely in different cell types. Interestingly, cells derived from human epithelia exposed to environmental pathogens, such as A549 human lung cells and CaCo-2 human colonic epithelial cells, showed the greatest levels of 3OC12-HSL inactivation. One can speculate that AHL inactivation occurs primarily in cells likely to come in contact with bacteria, and that this activity might play a role in the host innate defenses against pathogens.

The combined studies make a compelling case that bacterial autoinducers can modulate gene expression in host cells. However, it remains unclear as to whether these AHLs actually enter mammalian cells or exert their effects by binding to host cell surface receptors. This question was recently addressed in a report by Williams et al. (2004), who demonstrated that P. aeruginosa 3OC12-HSL can both enter mammalian cells and activate chimeric transcriptional factors based on its cognate LasR transcriptional activator. The autoinducer promoted nuclear localization of the chimeric LasR, and these LasR-based proteins activated the transcription of reporter fusions containing LasR-DNA target sequences. Although specific mammalian receptor proteins for bacterial autoinducers remain to be identified, the observation that the TraR autoinducer-binding domain resembles a GAF or PAS domain (involved in small molecule sensing in mammalian signaling proteins) might offer clues for possible autoinducer targets within mammalian cells (Vannini et al., 2002). In summary, eukaryotes seem to have a range of functional responses to AHLs that may play important roles in the beneficial or pathogenic outcomes of eukaryote–prokaryote interactions.

Quorum sensing in Gram-positive organisms

Quorum sensing in Gram-positive organisms relies on autoinduction by small peptides, which interact with two-component systems ultimately regulating gene transcription. These small peptides are usually products of oligopeptides that are cleaved and/or further modified before being exported from the bacterium by transporters. At threshold concentrations, the peptides are recognized by sensor kinases that initiate phospho-transfer to a response regulator. The peptides involved in Gram-positive QS are often specific for their cognate receptors. The following are several important examples of Gram-positive systems.

Staphylococcus aureus is one of the most common commensal Gram-positive organism in humans; however, it can lead to pneumonia, endocarditis, osteomyelitis, wound infections, and other complications. The QS system that this bacterium utilizes is one of the most studied systems in Gram-positive organisms. The accessory gene regulator (Agr) system regulates toxin and protease secretion in staphylococci. At low cell density, the bacteria express proteins required for attachment and colonization, and as the cell density becomes higher, this expression profile...
switches to express proteins involved in toxin and protease secretion (Novick, 2003).

The *S. aureus* autoinducing peptide (AIP) is encoded by the *agrD* gene. AgrB then adds a thiolactone ring to this peptide and transports the AIP out of the cell. The AIP works with its receptor, sensor kinase ArgC and ArgC’s cognate response regulator, ArgA. Upon AIP binding to ArgC, ArgC transfers a phosphate to ArgA, which activates transcription of the *arg* operon for autoregulation and in addition activates transcription of the RNAIII, regulatory RNA, which in turn leads to the repressed expression of cell adhesion factors and induced expression of secreted factors (Novick, 2003).

The specificity of the Arg system is such that, not only will noncognate AIP and receptors not specifically recognize each other, but each AIP will also repress the other AIPs by competitive binding to noncognate receptors. Thus far, there are four known specificity groups that have been characterized by sequence variation, and each group seems to result in a different disease. For example, *S. aureus* group III seems to correlate with menstrual toxic shock syndrome, type III with necrotizing pneumonia, and type Ia and type IIs have been associated with vancomycin resistance. Thus, in coinfection models, one group will out-compete the other leading to only one disease progression. This might have allowed evolution of *S. aureus* into suited environments (Novick, 2003).

*Enterococcus faecalis* are commensal organisms of the gastrointestinal tract that are often seen in nosocomial infections like surgical infections and urinary tract infections. Cytolysin, the major virulence factor in *E. faecalis*, possesses both bacteriocin and hemolytic activity, making it lethal to many eukaryotic cells as well as toxic to many Gram-positive bacteria. The cytolysin also serves as an autoinducer for QS induction of the cytolysin operon. Composed of two subunits, CyLL and CyLS, which are posttranslationally modified in order to form their mature extracellular form, control of the cytolysin is regulated by a threshold concentration of the mature form of CyLS (Haas et al., 2002). CyLL has been shown to bind strongly to target cell membranes, allowing free CyLSs to accumulate above a critical induction threshold (Goburn et al., 2004). This subunit acts as an autoinducer that activates transcription from the *cyl* promoter creating high levels of cytolysin when conditions are appropriate. CylR1 and CylR2 are genes transcribed from the *cyl* genes, and mutation in either one leads to derepression of the *cyl* operon. They comprise a two-component system, although lacking similarity to two-component regulators (Haas et al., 2002).

*Streptococcus pneumoniae* was one of the original Gram-positive systems characterized. Only under certain conditions, i.e. high cell density, was *S. pneumoniae* capable of competence (Tomasz & Hotchkiss, 1964; Tomasz, 1965; Tomasz & Mosser, 1966). Subsequently, an activator was discovered that was capable of inducing competence in *S. pneumoniae* as a substitute for actual cells. While original characterization of the activator proved difficult, the discovery of an ABC transporter that secreted the activator, *comA*, revealed a class of ABC transporters that contained N-terminal proteolytic domains (Hui & Morrison, 1991; Havarstein et al., 1995; Zhou et al., 1995). This led to the characterization of the activator as competence inducing factor (CSP), a cationic, and 17 amino acid peptide that contains a gly–gly leader sequence, which is cleaved from the original precursor (Havarstein et al., 1995).

The structural gene of CSP, *comC*, lies in an operon with *comD*, which encodes a histidine kinase, and *comE*, encoding a response regulator (Pestova et al., 1996). *ComD* serves as the receptor for CSP (Havarstein et al., 1996). Currently, 10 different *comD* alleles that encode different ComD specificities, primarily in the N-terminal regions, have been identified. When phosphorylated, ComE activates the *comCDE* operon in autoregulatory fashion in addition to *comAB* and the *comX*, a regulator that induces genes involved in competence. In addition, ComE induces genes involved in stress responses and protein synthesis (Lee & Morrison, 1999; Peterson, 2004).

Competence inducing factors have been shown to be highly specific, so that a CSP from one species will only specifically recognize its own cognate histidine kinase receptor. This specificity means that often even different isolates of the same species have distinctions (Havarstein et al., 1997).

Although the CSP–QS system in streptococci is a classic example of competence regulation, in a recent transcriptome analysis, a small fraction of the 124 genes induced by the CSP system are actually required for transformation. This indicates that the CSP system might be involved in other types of regulation. Indeed, initial studies have already implicated the importance of the CSP–QS system in adaptive environments and for functions such as virulence and biofilm formation (Cvitkovitch et al., 2003; Peterson, 2004).

The LuxS/AI-2 signaling system

*Vibrio harveyi* is a marine bacterium that controls bioluminescence through QS. *Vibrio harveyi* QS system constitutes a mix between components of Gram-positive and Gram-negative systems. It has two QS systems: system 1 in which the autoinducer (AI-1) is an AHL, and is primarily involved in intraspecies signaling (Bassler et al., 1994); system 2, in which the autoinducer is a furanosyl borate diester (Chen et al., 2002) involved in interspecies signaling (Surette & Bassler, 1998). *Vibrio harveyi* has two hybrid sensor kinases, LuxN and LuxQ, which sense AI-1 and AI-2, respectively. In the absence of signal, these proteins are intrinsic kinases and
phosphorylate a complex phosphorelay system, with LuxU and LuxO (an enhancer-binding protein) as intermediaries (Bassler et al., 1993, 1994; Freeman & Bassler, 1999; Freeman et al., 2000). Phospho-LuxO in conjunction with $\sigma^S$ then activates transcription of small regulatory RNAs, which destabilize the message of the LuxR protein, which in turn no longer can activate transcription of the luciferase operon (Lenz et al., 2004). Upon interaction with their cognate autoinducers, these sensors behave as phosphatases and the system is dephosphorylated, allowing LuxR to activate bioluminescence (Fig. 2).

Whether AI-1 directly interacts with LuxN remains to be demonstrated. The AI-2 receptor is the periplasmic protein LuxP, which resembles the ribose-binding protein RbsB (Chen et al., 2002). LuxP complexes with LuxQ controlling whether LuxQ behaves as a kinase or phosphatase according to the concentration of AI-2 present (Neiditch et al., 2005). The AI-1 synthase is the LuxM gene, which does not belong to the same family of the LuxI-type proteins (Bassler et al., 1993). AI-2 is synthesized by the LuxS enzyme (Surette & Bassler, 1998; Surette et al., 1999). LuxS is an enzyme involved in the metabolism of SAM; it converts S-ribosylhomocysteine into homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD). DPD is a very unstable compound that reacts with water and cyclizes to form several furanones (Schauder et al., 2001; Winzer, 2002; Sperandio et al., 2003), one of which is thought to be the precursor of AI-2 (Schauder et al., 2001). The AI-2 structure has been solved by cocrystallizing this ligand with its receptor LuxP in V. harveyi and reported to be a furanosyl borate diester (Chen et al., 2002). However, LuxP homologs, as well as homologs from this signaling cascade, have only been found in Vibrio sp. Several bacterial species harbor the luxS gene, and have AI-2 activity as measured using a V. harveyi bioluminescence assay (Schauder & Bassler, 2001; Xavier & Bassler, 2003). However, the only genes shown to be regulated by AI-2 in other species encode for an ABC transporter in Salmonella typhimurium named Lsr (LuxS-regulated), responsible for the AI-2 uptake (Taga et al., 2001). This ABC transporter is also present in E. coli and shares homology with sugar transporters. Once inside the cell, AI-2 is modified by phosphorylation and proposed to interact with LsrR, which is a SorC-like transcription factor involved in repressing expression of the lsr operon (Taga et al., 2001, H...
Several groups have been unable to detect the furanosyl borate diester, proposed to be AI-2, in purified fractions containing AI-2 activity from Salmonella and E. coli sp. (as measured using the V. harveyi bioluminescence assay) (Schauder et al., 2001; Winzer, 2002; Sperandio et al., 2003). These fractions only yielded several furanones that did not contain boron. These results can be explained now that AI-2 has been cocrystallized with its receptor (the periplasmic protein LsrB) in Salmonella. In these studies the LsrB ligand was not the furanosyl borate diester, but 2R, 4S-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (Miller et al., 2004), consistent with what has been observed in AI-2 fractions of Salmonella and E. coli (Schauder et al., 2001; Winzer, 2002; Sperandio et al., 2003). This scenario is fundamentally different from AI-2 detection in V. harveyi, and raises the question whether all bacteria may actually use AI-2 as a signaling compound, or whether it is released as a waste product or used as a metabolite by some bacteria, rather than as a signal (Winzer, 2002; Winzer et al., 2002).

Diverse roles in signaling have been attributed to AI-2 in other organisms by comparing luxS mutants with wild-type strains and complementing these mutants either genetically or with spent supernatants (Xavier & Bassler, 2003). Several of these studies comprised transcriptome analysis measuring genes differentially expressed between wild-type strains and the luxS mutants. Given that LuxS is not devoted to AI-2 production, it is in fact an enzyme involved in the biochemical pathway for detoxification of SAM. Consequently, altered gene expression because of a luxS mutation will comprise both genes affected by QS per se and genes differentially expressed because of the interruption of this metabolic pathway. To address which genes are in fact regulated through AI-2 signaling, one has to use pure AI-2 signal. Hence, the only two phenotypes shown to be AI-2 dependent, using either purified or in vitro synthesized AI-2, are bioluminescence in V. harveyi (Schauder et al., 2001) and expression of the lsr operon in S. typhimurium (Taga et al., 2001).

The AI-3/epinephrine/norepinephrine signaling system

This QS system was first discovered by serendipity as being associated with the LuxS system. LuxS is not devoted solely to AI-2 production; it is in fact an enzyme involved in the activated methyl pathway which is involved in the synthesis of methionine and SAM. Consequently, altered gene expression because of a luxS mutation will involve genes affected by QS per se and genes differentially expressed because of the interruption of this metabolic pathway. A luxS mutant will accumulate S-ribosyl-homocysteine within the cell because it is unable to catalyze its conversion to homocysteine. This would cause the levels of homocysteine to diminish within the cell. Inasmuch as homocysteine is used for the de novo synthesis of methionine, the cell will use a salvage pathway. It will use oxaloacetate to produce homocysteine to synthesize methionine. Given that oxaloacetate and L-glutamate are necessary to synthesize aspartate, using this salvage pathway for the de novo synthesis of methionine, other amino acid synthetic and catabolic pathways will be changed within the cell (http://www.ecosal.org/ecosal/index.jsp). Changes in other amino acid metabolic processes are responsible for the lack of AI-3 activity in a luxS mutant. Hence, LuxS is not involved in the synthesis of AI-3 per se (M. Walters & V. Sperandio, unpublished results). Structural analysis of AI-3 suggests that this signal is an aromatic compound and does not contain a sugar skeleton like AI-2 (J.R. Falk & V. Sperandio, unpublished data).

It has recently been shown, using anaerobically cultured stools from healthy human volunteers, that the microbial intestinal flora produce AI-3 (Sperandio et al., 2003). To obtain further information regarding which intestinal commensals and pathogens produce AI-3, freshly isolated strains from patients were tested (M.P. Sircili & V. Sperandio, unpublished observations). AI-3 activity was observed in spent supernatants from enteropathic E. coli strains from serogroups O26:H11 and O111ac:H9, Shigella sp, and Salmonella sp. AI-3 activity was also detected in normal flora bacteria such as commensal E. coli, Klebsiella pneumoniae, and Enterobacter cloacae (M.P. Sircili & V. Sperandio, unpublished observations). These results suggest that AI-3 production is not limited to EHEC and that AI-3 may be involved in interspecies signaling among intestinal bacteria. Besides being used in bacterial interspecies signaling, AI-3 has an intrinsic role in interkingdom communication. AI-3 cross signals with the eukaryotic hormones epinephrine/norepinephrine in an agonistic fashion (Sperandio et al., 2003). Both epinephrine and norepinephrine are present in the gastrointestinal (GI) tract. Norepinephrine is synthesized within the adrenergic neurons present in the enteric nervous system (ENS) (Furness, 2000). Although epinephrine is not synthesized in the ENS, being synthesized in the central nervous system (CNS) and in the adrenal medulla, it acts in a systemic manner after being released by the adrenal medulla into the bloodstream, thereby reaching the intestine (Furness et al., 2001). Both hormones modulate intestinal smooth muscle contraction, submucosal blood flow, and chloride and potassium secretion in the intestine (Horger et al., 1998). Consequently, being able to monitor the level of both these hormones in the intestine might aid bacteria to gauge the metabolic state of the host. There are currently nine known human adrenergic receptors, partitioned into three subclasses: α1, α2, and β. Fredolini et al. (2004) recently reported the 3D structure of human β2 adrenergic receptor, and predicted that the ligand-binding sites for epinephrine...
and norepinephrine are broadly similar. Taken together, there is extensive evidence in the literature that both epinephrine and norepinephrine are recognized by the same receptors, and that both catecholamines have important biological roles in the human GI tract. This signaling system activates transcription of several virulence genes in EHEC.

Enterohemorrhagic E. coli O157:H7 is responsible for major outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) throughout the world. EHEC has a very low infectious dose (as few as 50 cfu), which is one of the major contributing factors to EHEC outbreaks. Treatment and intervention strategies for EHEC infections are still very controversial, with conventional antibiotics usually having little clinical effect and possibly even being harmful (by increasing the chances of patients developing HUS (Kaper et al., 2004)).

Enterohemorrhagic E. coli colonizes the large intestine where it causes attaching and effacing (AE) lesions. The AE lesion is characterized by the destruction of the microvilli and the rearrangement of the cytoskeleton to form a pedestal-like structure, which cups the bacteria individually. The genes involved in the formation of the AE lesion are encoded within a chromosomal pathogenicity island named the LEE (Jarvis et al., 1995). The LEE region contains five major operons: LEE1, LEE2, LEE3, tir (LEE5) and LEE4, which encode a type III secretion system (TTSS), an adhesin (intimin), and this adhesin's receptor (Tir), which is translocated to the epithelial cell through the bacterial TTSS (Elliott, 1998; Mellies et al., 1999). The LEE genes are directly activated by the LEE-encoded regulator (Ler), which is the first gene in the LEE1 operon (Mellies et al., 1999; Elliott, 2000; Sperandio et al., 2000; Bustamante et al., 2001; Sanchez-SanMartin et al., 2001). Transcription of the LEE genes is further positively and negatively modulated by GrlA and GrlR, respectively, which are encoded in a small operon downstream of LEE1 (Deng, 2004). EHEC also produces a potent Shiga toxin (Stx) that is responsible for the major symptoms of hemorrhagic colitis and HUS.

Enterohemorrhagic E. coli senses AI-3 (produced by the normal GI flora) and epinephrine/norepinephrine produced by the host to activate expression of the LEE genes and the flagella regulon (Sperandio et al., 2003). These signals are sensed by sensor kinases in the membrane of EHEC that relay this information through a complex regulatory cascade.

Fig. 3. Model of quorum sensing signaling in enterohemorrhagic Escherichia coli. Both AI-3 and epinephrine/norepinephrine seem to be recognized by the same receptor, which is probably in the outer membrane of the bacteria. These signals might be imported to the periplasmic space where they interact with two major sensor kinases. QseC might be the sensor kinase transducing these signals towards activation of the flagella regulon, whereas QseE might be the sensor kinase transducing these signals to activate transcription of the locus of enterocyte effacement (LEE) genes. QseC phosphorylates the QseB response regulator, which binds to the promoter of flhDC to activate expression of the flagella regulon. QseB also binds to its own promoter to positively autoregulate its own transcription. QseE is the sensor kinase and its predicted response regulator is QseF. At what levels QseF regulates transcription of the LEE genes remain to be established. QseA is one of the transcriptional factors involved in the regulation of ler (LEE1) transcription in two levels, by binding and activating transcription of LEE1 and by activating transcription of the grlIRA operon, where GrlA and GrlR positively and negatively regulate expression of ler, respectively. Then, in a cascade fashion, Ler activates transcription of the other LEE genes. QseD is a second LysR-like regulator, involved in modulating expression of the LEE and flagella genes.
that activates the flagella regulon and the LEE pathogenicity island. The sensor for the flagella regulon is QseC that autophosphorylates in response to both epinephrine and AI-3 and transfers its phosphate to the QseB response regulator, which in turn activates transcription of the flagella genes and itself (Sperandio, Torres et al., 2002; Clarke & Sperandio, 2005a, b) (M.B. Clarke & V. Sperandio, unpublished data). We recently identified a second two-component system named QseEF, which is essential for AE lesion formation (R. Reading & V. Sperandio, unpublished data).

Further regulation of the LEE genes is complex and requires at least two LysR transcription factors (QseA and QseD) (Sperandio, Li et al., 2002) (F. Sharp, M. Walters and V. Sperandio, unpublished data), which in concert with several global regulators in EHEC ensure the correct kinetics of LEE gene expression (Fig. 3). The AI-3/epinephrine/norepinephrine signaling cascade is present in several bacterial species (e.g. Shigella, Salmonella, Erwinia carotovora, Pasteurella multocida, Haemophilus influenzae, Actinobacillus pleuropneumoniae, Chromobacterium violaceum, Coxiella burnetii, Yersinia, Francisella tularensis andRalstonia solaecearum) suggesting that this interkingdom crosssignaling is not restricted to E. coli.

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References


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