Published in final edited form as:

Cell Microbiol. 2009 March ; 11(3): 363–369. doi:10.1111/j.1462-5822.2008.01272.x.

Cell-to-cell signalling during pathogenesis

Christopher T. Parker and **Vanessa Sperandio***

University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9048, USA.

Summary

Pathogenic bacteria produce virulence factors only when they sense they are in a location in which the energy required for pathogenesis is warranted. One environmental factor monitored by pathogens is population density, either of its own population or of the population of a host's endogenous flora. To date, four systems have been described which allow pathogens to regulate virulence genes in a population-dependent manner. These systems are found in both Gram-positive and Gram-negative cells and utilize various mechanisms to control gene regulation at a transcriptional level, but they all have one feature in common: they detect auto-inducers released by cells belonging to either the pathogen's population or that of the host's flora. This article explores the role of these four signalling systems in bacterial communities and how pathogens use these systems to control genes required during host invasion and infection.

Introduction

One of the tenets of microbiology is that bacteria sense changes in their environments and alter their behaviour in order to better survive. When a bacterium's environment changes, the response is often a programmed alteration in gene expression that results in a better chance at survival. Pathogens are no different. What we consider pathogenesis can easily be viewed as bacteria simply adapting their behaviour to survive, and thrive, in a new environmental niche: the host organism. This pathogenic response, however, comes at a cost to invading bacteria. During pathogenesis, numerous virulence factors, effector proteins and, occasionally, toxins are produced and used in various ways to help the bacteria survive within the host organism. Enterohaemorrhagic *Escherichia coli*, for example, uses type III secretion to translocate effector molecules to the cytoplasm of intestinal cells during colonization. *Brucella*, on the other hand, use type IV secretion to translocate effector molecules into the host cell that enable bacterial survival within macrophage vacuoles after phagocytosis. The bacteria in both of these cases affect their environment in such a way that they can survive where most bacteria would perish. However, the secretion machinery used by these bacteria is comprised of dozens of proteins that must first be assembled within the bacterial membrane before it can be used to translocate effector proteins across the bacterial and host membranes. The energy required to synthesize and maintain these molecular machines makes it impractical for them to be present when not needed. As a result, the genes responsible for these tasks, often found in pathogenicity islands or on plasmids, are tightly controlled at the transcriptional level and are not expressed until the bacteria sense they are in an environment in which the energy expense required for pathogenesis is justified. Once the appropriate stimulus is received, transcription factors direct the transcription of these operons.

The environmental stimulus that triggers pathogenesis can come in a number of forms: temperature, pH and host-derived molecules, to name a few. In addition to these factors contributed by the host, many pathogens can also detect signals originating from other bacteria. In the process known as quorum sensing, the bacterium produces a signal, called autoinducer, which is released into the environment. When the autoinducer reaches a critical concentration due to an increase in bacterial population, it triggers changes in gene expression resulting in a shift in the bacterium's life cycle and metabolism. In the case of some pathogenic bacteria, quorum sensing is used to detect when the community to which it belongs has reached a population large enough to make a virulent lifestyle worthwhile, such as when biofilm formation is required. However, it is becoming clear that autoinducer-based signalling systems that do not fit into the strict quorum sensing model are also used by pathogenic bacteria. An example of this is when pathogenic bacteria use signalling molecules to detect the presence of host flora, thereby indicating that the pathogen is in a location suitable for colonization. In such cases, the detected signal is not provided by the invading bacterium, but rather by an established, high-density population. As a result, the invading bacterium does not need to produce and release signal, only to detect it.

To date, there are four main categories of cell-to-cell signalling systems. Two of these systems, which use autoinducer-1 (AI-1) and autoinducer-3 (AI-3), are found in Gram-negative cells while the Gram-positive cells use an autoinducing polypeptide (AIP) system. The fourth system, using autoinducer-2 (AI-2), is found in Gram-positive and Gram-negative cells and might represent a generalized signalling system. All of these systems begin with production and release of autoinducer into the environment, either by the pathogen or by the resident flora. The detection of these chemically distinct autoinducers, and the resulting alteration in gene expression, is specific to each system.

AI-1/LuxI/R-based system

Since its initial discovery as the reason behind light production in *Vibrio fischeri*, the LuxI/LuxR system has become the model system of quorum sensing upon which the other systems have been based. It consists of LuxI, which synthesizes an *N*-acyl homoserine lactone (AHL) called AI-1, and LuxR, a transcription factor responsible for controlling gene expression in the presence of the autoinducer (Fig. 1A). LuxI and its homologues synthesize auto-inducers by transferring a fatty acid chain from an acylated acyl carrier protein (ACP) to *S*-adenosylmethionine (SAM), releasing the AHL and methylthioadenosine (Schaefer *et al.*, 1996). AHLs produced by LuxI homologues possess different fatty acid moieties due to recognition of specific ACPs by the synthases and results in genus- and species-specific signals. After synthesis by LuxI, AI-1 is free to diffuse across the bacterial membrane, thereby being released into the surrounding environment. Since each individual bacterium within the growing population is producing AI-1 simultaneously, the concentration of AI-1 in the environment rises as the population increases. At high population density, the local concentration of AI-1 is high enough to diffuse back into the cell, where LuxR then binds it. When bound to AI-1, LuxR activates the transcription the *luxCDABEGH* operon by binding Lux boxes located within the promoter (Devine *et al.*, 1989). The product of this operon, luciferase, catalyses a chemical reaction that results in luminescence.

Homologous LuxI/LuxR systems have been identified in many Gram-negative bacteria, each capable of producing specific AHLs. In some bacteria, such as *Pseudomonas aeruginosa* and *Serratia marcescens*, both opportunistic pathogens, these signalling mechanisms control pathways responsible for expressing various virulence factors. Both of these organisms are capable of forming biofilms on medical implants and catheters, possibly resulting in persistent infections in hospital patients. Due to the nature of biofilms, these infections are less susceptible to treatment by antibiotics than they would be normally as individual cells. *P. aeruginosa*

contains two systems homologous to LuxI/LuxR. LasI/LasR has been shown to control biofilm formation and the production of extracellular enzymes, as well as transcription of another quorum sensing system, RhlI/RhlR, adding an additional level of control through AHL signalling (De Kievit and Iglewski, 2000). Both systems play a role in virulence as *lasI* and *rhlI* strains, either as single or as double mutants, are less successful in colonizing the lungs of neonatal mice than the isogenic wild-type strain (Pearson *et al.*, 2000). *S. marcescens* also has a homologous LuxI/LuxR system. This system, in the presence of the *Serratia*-produced AHL, has been implicated in the control of numerous pathways linked to pathogenesis in *S. marcescens* clinical strain 12 (*Sma* 12). When compared with wild-type *Sma* 12, a strain lacking the AHL synthase *SmaI* showed decreased swarming motility, less cell adhesion (presumed to be a defect in biofilm formation), and reduced secretion of extracellular caseinase, chitinase and haemolysin (Coulthurst *et al.*, 2006). These defects were all corrected by addition of AHL, indicating that these traits are influenced, either directly or indirectly, through cell-to-cell signalling.

The intracellular pathogen *Brucella melitensis* uses AHL to regulate two macromolecular machines required during pathogenesis: a polar flagellum and a type IV secretion system (Delrue *et al.*, 2005). However, AHL regulation of the operons responsible for these machines differs from the prototypic LuxI/LuxR system described above. The *B. melitensis* LuxR-like transcriptional regulator, VjbR, activates genes associated with flagellar biosynthesis and type IV secretion in the absence of *Brucella*-specific AHL. In the presence of AHL, VjbR is inactive and the genes are expressed at a lower level. Therefore, an increase in population density results in decreased expression of these virulence factors. The role of these two macromolecular machines in pathogenesis is still unclear, but quorum sensing appears to play a role in coordinating the expression of these complexes either during the onset of infection or during the intracellular life cycle of the bacterium. Interestingly, although a *Brucella*-specific AHL has been identified, the *Brucella* chromosome does not contain a LuxI homologue.

Although early studies suggested that these systems were designed to monitor the population density of an organism's own population, it is becoming increasingly clear that this is not always the case. *E. coli* and *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*), for example, do not have a LuxI homologue and therefore do not produce any AI-1, but they do encode a LuxR homologue named SdiA that when overexpressed has been shown to have a negative effect on genes involved in cell attachment in enterohaemorrhagic *E. coli* (EHEC) (Kanamaru *et al.*, 2000), but positively regulates several genes located on the *S. Typhimurium* virulence plasmid including *rck*, a protein implicated in evasion of the host immune response (Ahmer *et al.*, 1998). Although the exact role SdiA plays in pathogenesis is unclear, this protein allows EHEC and *S. Typhimurium* to alter gene expression in response to the presence of AI-1 produced by other bacteria (Michael *et al.*, 2001). This would be of particular use to these two pathogens since they both colonize within the gastrointestinal (GI) tract where AI-1, produced by host's natural flora, may be present.

AI-2/LuxS-based system

Although considered the prototypical mechanism for cell-to-cell signalling, the AI-1 system is not the only strategy available. Many bacteria, Gram-negative and Gram-positive alike, have a system that detects an extracellular signal named autoinducer-2 (AI-2). This signal is different from the AHLs described above and is synthesized from a by-product of SAM metabolism. LuxS converts ribosehomocysteine into homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), a compound that, in the presence of water, cyclizes into several furanones (Schauder *et al.*, 2001). The structure of two AI-2 signals have been determined by co-crystallization with two different AI-2-binding proteins: a furanosyl borate diester used by *Vibrio harveyi* to control

luminescence, and a furanone [(2R,4SL)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF)] used by *S. Typhimurium* (Chen *et al.*, 2002; Miller *et al.*, 2004).

Like AI-1, AI-2 in either form is released by the bacterium where it accumulates in the cell's environment. Detection of AI-2, however, is different from AI-1 detection. In fact, two separate mechanisms resulting in the detection of AI-2 have been described. The first, described in *V. harveyi*, is specific to the borate diester form of AI-2. This mechanism detects the presence of AI-2 in the periplasm by first binding the signal with an autoinducer-specific binding protein, LuxP. This AI-2/LuxP complex then interacts with a sensor kinase, LuxQ, initiating a phospho-transfer cascade that results, through deactivation of the negative response regulator LuxO, in luciferase production and luminescence (Fig. 1B). In pathogenic *Vibrio cholerae* LuxO is believed to repress the production of transcriptional regulator HapR at low cell density, which in turn represses production of several virulence factors (Zhu *et al.*, 2002). As a result, this bacterium has the counterintuitive trait of expressing virulence factors at low population density while down-regulating these virulence factors as the level of autoinducer increases.

To date, the LuxP/LuxQ cascade has only been identified in *Vibrio* spp. AI-2 is handled by a different mechanism in *E. coli* and *S. Typhimurium*. These organisms import AI-2 by a mechanism homologous to ribose import by the *rbs* system. Unlike the LuxP/LuxQ system, the Lsr (LuxS-regulated) system induces a cellular response by transporting AI-2 into the cytoplasm of the cell. This process starts with recognition of the signal by a periplasmic protein, LsrB, which binds the R-THMF form of AI-2. Once bound, the Lsr ABC transporter, comprised of LsrA and LsrC, imports AI-2 into the cell where LsrK phosphorylates it. It is believed that the phosphorylated form of AI-2 interacts with the transcriptional repressor LsrR to relieve repression of the *lsr* operon (Taga *et al.*, 2001). Although it has not been shown directly, it is possible that this mechanism, or one similar to it, might result in the upregulation of additional operons in the presence of phosphorylated AI-2.

LuxS/AI-2 systems have been discovered in a wide range of Gram-positive and Gram-negative bacteria leading to the suggestion that the AI-2 system represents a method of cross-species signalling (Xavier and Bassler, 2003). Considering the mixed nature of most natural bacterial communities, such as that found in the GI tract of mammals, this common signal might be used to regulate genes needed to survive in the presence of other bacteria. Along these same lines, pathogenic bacteria may use these same signals to activate genes that would allow it to gain an advantage in this same environment. However, the role of AI-2 in non-*Vibrio* pathogenesis is unclear. Early studies using either *luxS* mutants or preconditioned media reported increased expression of flagella and type III secretion machinery in EHEC only under conditions where autoinducer was present (Sperandio *et al.*, 1999; 2001). However, it should be noted that a direct influence on these genes by phosphorylated AI-2 or an AI-2-bound response regulator was never shown. In fact, the discovery of a third autoinducer, described below in more detail, presents the possibility that upregulation of these genes was due to a compound other than AI-2 in the preconditioned media. The data from the *luxS* mutant might have been misleading, as well. Because LuxS is part of the pathway responsible for synthesis of SAM and methionine, a *luxS* mutant would be at a metabolic disadvantage compared with the wild-type strain. The lack of available methionine in the cell caused by this break in a metabolic pathway might have pleiotropic effects caused by unreliable protein translation (Winzer *et al.*, 2002).

AI-3/QseC system

Of the three Gram-negative cell-to-cell signalling systems described to date, the AI-3 system is the least studied. AI-3 was first described as a compound found in spent media, different from AI-2, which activated the expression of genes involved in attachment of EHEC to, and subsequent actin rearrangement in, eukaryotic cells (Sperandio *et al.*, 2003). The structure and

synthesis of this signal is unclear and might, in fact, represent a family of molecules as AI-1 does. Early data suggested that LuxS was, once again, involved in some way because AI-3 production is impaired in *luxS* mutants. Further studies, however, proved that the lack of AI-3 production in these mutants was caused by a shift in the cell's metabolism as it uses oxaloacetate, instead of SAM, as a precursor for methionine and that addition of L-aspartate to the growth medium, thereby relieving the demand for oxaloacetate, restored AI-3 production but had no effect on AI-2 production (Walters *et al.*, 2006). This study also found that a number of commensal bacteria such as non-pathogenic *E. coli* and *Enterobacter cloacae*, as well as pathogenic *Shigella*, *Salmonella* and *Klebsiella* species, all produce AI-3. This suggests that AI-3 might represent another cross-species signal but, unlike AI-2, this new signal has not yet been detected in Gram-positive bacteria. However, the role of AI-3 in commensal communities has not yet been determined.

Detection of AI-3 is accomplished through a two-component system comprised of the sensor kinase QseC and response regulator QseB (Fig. 1C). In the presence of periplasmic AI-3, QseC first undergoes autophosphorylation and then transfers this phosphate to QseB, which activates genes responsible for flagella biosynthesis and motility by upregulating the master flagellar regulator genes *flhDC* (Clarke *et al.*, 2006). As mentioned above the presence of AI-3 is also linked to the formation of attaching and effacing (AE) lesions by EHEC, a feat accomplished through upregulation of five separate loci of enterocyte effacement (LEE) operons located within the EHEC chromosome (Sperandio *et al.*, 2003). The complete cascade responsible for regulation of these genes remains unclear, but likely involves QseA, a LysR-family regulator that is influenced by cell-to-cell signalling and directly upregulates LEE genes (Sperandio *et al.*, 2002). Based on these observations, it has been proposed that enteric pathogens might use AI-3 produced by the host's flora as an indicator that they are in a habitable location in the GI tract and that upregulation of flagella and motility are needed to penetrate the mucosal lining of the colon in order to reach underlying epithelial cells. Once in contact with epithelial cells, proteins encoded by the LEE genes enable the pathogen to attach to the eukaryotic cells for the purpose of colonization.

Intriguingly, the QseBC cascade also responds to the adrenergic signals adrenaline and noradrenaline, both found in the GI tract (Clarke *et al.*, 2006). This suggests that QseC responds to bacterial and host signals simultaneously. Conversely, this presents the possibility that adrenergic receptors on eukaryotic cells might respond to AI-3 as well as adrenaline or noradrenaline. If this is the case, enteric bacteria might take advantage of this communication to prepare epithelial cells of the host GI tract for colonization, thereby preconditioning the GI tract for subsequent colonization by pathogens.

AIP/Agr system

This cell-to-cell signalling system, found exclusively in Gram-positive bacteria, is based on the prototypic Agr system first identified in *Staphylococcus aureus*. Unlike the previously described systems, the Gram-positive system uses a polypeptide signal instead of a smaller molecule (Fig. 2). These polypeptides play a dual role: they act as an autoinducer for the organism that produced it but an inhibitor to other organisms. This signal is referred to as an AIP and is encoded by the *agrD* gene. After translation, the AgrD propeptide is targeted to the membrane by an N-terminal signal sequence. Once at the membrane, AgrB, a membrane-bound endopeptidase, cleaves the C-terminus of the propeptide. The N-terminus of the propeptide, including the signal sequence, is removed by the signal peptidase SpsB. Finally, the C-terminus of this processed polypeptide is covalently linked to a conserved, centrally located cysteine to form a thiolactone ring with a free N-terminal tail. In many cases, both of these structures are required for proper function of the AIP. After release into the environment, the AIP is recognized by a signal receptor, AgrC. This protein contains an N-terminal transmembrane

domain responsible for recognizing specific AIPs and a C-terminal histidine kinase domain that, in the presence of the correct AIP, phosphorylates a response regulator called AgrA. Phosphorylated AgrA activates transcription of select genes by binding direct repeats located in the promoter regions. One aspect that is unique to the AIP/Agr system is the fact that an AIP produced by one strain of *Staphylococcus* will simultaneously interfere with another strain's Agr system. This dual role as activator and inhibitor is related to the interaction between the AIP and AgrC. The cyclic structure of AIP is required for interaction with AgrC, but it is the N-terminal tail that is responsible for AgrC activation. Removal of this tail, in fact, results in a universal inhibitor that binds to AgrC but is unable to activate the Agr system (Lyon *et al.*, 2000).

The Agr system has been linked to pathogenesis in numerous Gram-positive organisms. For example, *S. aureus* strains lacking the Agr system are impaired in their ability to cause osteomyelitis in both rabbit and murine models (Gillaspy *et al.*, 1995; Blevins *et al.*, 2003). AIP-directed regulation of genes by AgrA results in production and release of numerous toxins by *S. aureus* such as alpha-, beta- and delta-haemolysins, serine proteases and toxic shock syndrome toxin 1 (TSST-1). The release of haemolysin and protease disrupts localized eukaryotic cells and aids in *Staphylococcal* adhesion whereas TSST-1 is a superantigen that binds non-specifically to T-cell receptors ultimately resulting in a strong immunological response due to systemic release of cytokines. In addition to upregulation of toxins, however, high concentration of AIP is also responsible for downregulation of surface-exposed proteins such as fibronectin-binding protein and protein A, two proteins involved in *Staphylococcal* adhesion. This reduction in cell adhesion would have a negative effect on the architecture of any biofilm-associated communities. As a result, the bacterial cells are released from their communities and are free to migrate to other locations within the host. Once a new, suitable location is found, the cell can reattach and begin multiplying again, possibly resulting in a secondary infection.

Enterococcus faecalis is another Gram-positive cell that benefits from the use of AIP signal sensing. This organism uses a two-component system homologous to Agr system of *Staphylococcus* to detect the presence of AIP. When AIP is detected, the cell produces and releases two extracellular proteases: gelatinase (GelE) and serine protease (SprE) (Qin *et al.*, 2000). The role of these two proteins in pathogenesis is still unclear, but gelatinase has been implicated in cell migration within hosts. In an *in vitro* assay measuring *E. faecalis* translocation across a monolayer of polarized human colon cells, the presence of gelatinase was found to be important (Zeng *et al.*, 2005). Translocation of *E. faecalis* across the epithelial cells of the colon might allow this organism to enter the vascular or lymphatic systems and migrate to other locations within the host where secondary infections might occur.

Interestingly, the AIP-based system is not the only quorum sensing system used by *E. faecalis*. Cytolysin production by this bacterium is repressed at a transcriptional level by two proteins: a membrane-bound sensor, CylR1, and a DNA-binding protein, CylR2 (Haas *et al.*, 2002). When the concentration of cytolysin outside the cell is low, CylR2 represses the genes encoding the cytolysin subunits, *cylL_L* and *cylL_S*, by binding the *cylL* promoter. CylR1 monitors the concentration of cytolysin outside the cell and when a threshold concentration is reached, CylR1 alters the DNA-binding capacity of CylR2. This results in the release of CylR2 from the *cylL* promoter and an increase in cytolysin production. As a result, cytolysin is upregulated in a population-dependent manner through its own detection rather than the through the detection of AIP.

Concluding remarks

It is becoming more and more clear that interbacterial communication plays an important role in bacterial interactions with host organisms. This is true for both natural, symbiotic flora and invading pathogenic bacteria. In the case of host flora, signalling molecules are used to monitor population densities to better coordinate population-wide responses, often resulting in the formation of complex biofilms. Some pathogens also use signalling molecules to coordinate population-dependent responses in cases where large number of bacteria are required for colonization, but can also use signal molecules produced by other populations, such as the existing flora, as an indicator of their location within the host. Using this strategy, invading bacteria switch on pathogenic genes only when they are in an environment where pathogenesis is likely to succeed. This insures that the bacterium does not expend the extra energy required for pathogenesis unless it is beneficial to do so.

Acknowledgments

We would like to apologize to any researchers whose work was not included in this review due to space limitations. Vanessa Sperandio is a Burroughs Wellcome Fund Investigator in the field of pathogenesis of infectious diseases. The work performed by the Sperandio lab was funded by NIH Grants AI053067 and AI07785301.

References

- Ahmer BM, van Reeuwijk J, Timmers CD, Valentine PJ, Heffron F. *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. *J Bacteriol* 1998;180:1185–1193. [PubMed: 9495757]
- Blevins JS, Elasri MO, Allmendinger SD, Beenken KE, Skinner RA, Thomas JR, Smeltzer MS. Role of *sarA* in the pathogenesis of *Staphylococcus aureus* musculoskeletal infection. *Infect Immun* 2003;71:516–523. [PubMed: 12496203]
- Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczar I, Bassler BL, Hughson FM. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 2002;415:545–549. [PubMed: 11823863]
- Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. The QseC sensor kinase: a bacterial adrenergic receptor. *Proc Natl Acad Sci USA* 2006;103:10420–10425. [PubMed: 16803956]
- Coulthurst SJ, Williamson NR, Harris AK, Spring DR, Salmond GP. Metabolic and regulatory engineering of *Serratia marcescens*: mimicking phage-mediated horizontal acquisition of antibiotic biosynthesis and quorum-sensing capacities. *Microbiology* 2006;152:1899–1911. [PubMed: 16804166]
- De Kievit TR, Iglewski BH. Bacterial quorum sensing in pathogenic relationships. *Infect Immun* 2000;68:4839–4849. [PubMed: 10948095]
- Delrue RM, Deschamps C, Léonard S, Nijskens C, Danese I, Schaus JM, et al. A quorum-sensing regulator controls expression of both the type IV secretion system and the flagellar apparatus of *Brucella melitensis*. *Cell Microbiol* 2005;7:1151–1161. [PubMed: 16008582]
- Devine JH, Shadel GS, Baldwin TO. Identification of the operator of the *lux* regulon from the *Vibrio fischeri* strain ATCC7744. *Proc Natl Acad Sci USA* 1989;86:5688–5692. [PubMed: 2762291]
- Gillaspy AF, Hickmon SG, Skinner RA, Thomas JR, Nelson CL, Smeltzer MS. Role of the accessory gene regulator (*agr*) in pathogenesis of staphylococcal osteomyelitis. *Infect Immun* 1995;63:3373–3380. [PubMed: 7642265]
- Haas W, Shepard BD, Gilmore MS. Two-component regulator of *Enterococcus faecalis* cytolysin responds to quorum-sensing autoinduction. *Nature* 2002;415:84–87. [PubMed: 11780122]
- Kanamaru K, Kanamaru K, Tatsuno I, Tobe T, Sasakawa C. SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol* 2000;38:805–816. [PubMed: 11115115]

- Lyon GJ, Mayville P, Muir TW, Novick RP. Rational design of a global inhibitor of the virulence response in *Staphylococcus aureus*, based in part on localization of the site of inhibition to the receptor-histidine kinase, AgrC. *Proc Natl Acad Sci USA* 2000;97:13330–13335. [PubMed: 11087872]
- Michael B, Smith JN, Swift S, Heffron F, Ahmer BM. SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. *J Bacteriol* 2001;183:5733–5742. [PubMed: 11544237]
- Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM. *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Mol Cell* 2004;15:677–687. [PubMed: 15350213]
- Pearson JP, Feldman M, Iglewski BH, Prince A. *Pseudomonas aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. *Infect Immun* 2000;68:4331–4334. [PubMed: 10858254]
- Qin X, Singh KV, Weinstock GM, Murray BE. Effects of *Enterococcus faecalis* *fsr* genes on production of gelatinase and a serine protease and virulence. *Infect Immun* 2000;68:2579–2586. [PubMed: 10768947]
- Schaefer AL, Val DL, Hanzelka BL, Cronan JE, Greenberg EP. Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *Proc Natl Acad Sci USA* 1996;93:9505–9509. [PubMed: 8790360]
- Schauder S, Shokat K, Surette MG, Bassler BL. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol Microbiol* 2001;41:463–476. [PubMed: 11489131]
- Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA* 1999;96:15196–15201. [PubMed: 10611361]
- Sperandio V, Torres AG, Girón JA, Kaper JB. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J Bacteriol* 2001;183:5187–5197. [PubMed: 11489873]
- Sperandio V, Li CC, Kaper JB. 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* 2002;70:3085–3093. [PubMed: 12011002]
- Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB. Bacteria-host communication: the language of hormones. *Proc Natl Acad Sci USA* 2003;100:8951–8956. [PubMed: 12847292]
- Taga ME, Semmelhack JL, Bassler BL. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. *Mol Microbiol* 2001;42:777–793. [PubMed: 11722742]
- Walters M, Sircili MP, Sperandio V. AI-3 synthesis is not dependent on *luxS* in *Escherichia coli*. *J Bacteriol* 2006;188:5668–5681. [PubMed: 16885435]
- Winzer K, Hardie KR, Burgess N, Doherty N, Kirke D, Holden MT, et al. LuxS: its role in central metabolism and the *in vitro* synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. *Microbiology* 2002;148:909–922. [PubMed: 11932438]
- Xavier KB, Bassler BL. LuxS quorum sensing: more than just a numbers game. *Curr Opin Microbiol* 2003;6:191–197. [PubMed: 12732311]
- Zeng J, Teng F, Murray BE. Gelatinase is important for translocation of *Enterococcus faecalis* across polarized human enterocyte-like T84 cells. *Infect Immun* 2005;73:1606–1612. [PubMed: 15731060]
- Zhu J, Miller MB, Vance RE, Dziejman M, Bassler BL, Mekalanos JJ. Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*. *Proc Natl Acad Sci USA* 2002;99:3129–3134. [PubMed: 11854465]

Fig. 1.

Three autoinducing strategies in Gram-negative cells.

A. AI-1-based strategy using acylated homoserine lactones (red diamonds). AI-1 is synthesized by LuxI and released into the environment. As AI-1 diffuses back into the cell, it interacts with LuxR and results in a cellular response.

B. AI-2-based strategy using either R-THMF (green pentagons) or furanosyl borate diester (blue pentagons), both created from LuxS-derived DPD. In *Vibrio* spp., furanosyl borate diesters interact with LuxP in the periplasm and initiates the LuxQ/LuxO phosphoryl cascade, but in other organisms R-THMF is imported into the cell and phosphorylated by the Lsr system.

C. AI-3-based strategy using an autoinducer of unknown structure (yellow hexagons). Autoinducer is released into the environment and sensed by QseC as it diffuses back into the periplasm. QseC then phosphorylates the response regulator QseB.

IM, inner membrane; OM, outer membrane.

Fig. 2. AIP-based autoinduction and inhibition in Gram-positive cells using polypeptides (green hexagons). Propeptide, encoded by AgrD, is processed by AgrB and released into the environment where it can either activate the AgrA/AgrC cascade or inhibit the same cascade of a different species. M, membrane; P, peptidoglycan.