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Bacterial Quorum Sensing: Its Role in Virulence and Possibilities for Its Control

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Quorum sensing is a process of cell–cell communication that allows bacteria to share information about cell density and adjust gene expression accordingly. This process enables bacteria to express energetically expensive processes as a collective only when the impact of those processes on the environment or on a host will be maximized. Among the many traits controlled by quorum sensing is the expression of virulence factors by pathogenic bacteria. Here we review the quorum-sensing circuits of *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa,* and *Vibrio cholerae*. We outline these canonical quorum-sensing mechanisms and how each uniquely controls virulence factor production. Additionally, we examine recent efforts to inhibit quorum sensing in these pathogens with the goal of designing novel antimicrobial therapeutics.

Quorum sensing (QS) is a bacterial cell–cell communication process that involves the production, detection, and response to extracellular signaling molecules called autoinducers (AIs). AIs accumulate in the environment as the bacterial population density increases, and bacteria monitor this information to track changes in their cell numbers and collectively alter gene expression. QS controls genes that direct activities that are beneficial when performed by groups of bacteria acting in synchrony. Processes controlled by QS include bioluminescence, sporulation, competence, antibiotic production, biofilm formation, and virulence factor secretion (reviewed in Novick and Geisinger 2008; Ng and Bassler 2009; Williams and Camara 2009).

Despite differences in regulatory components and molecular mechanisms, all known QS systems depend on three basic principles. First, the members of the community produce AIs, which are the signaling molecules. At low cell density (LCD), AIs diffuse away, and, therefore, are present at concentrations below the threshold required for detection. At high cell density (HCD), the cumulative production of AIs leads to a local high concentration, enabling detection and response (Kaplan and Greenberg 1985). Second, AIs are detected by receptors that exist in the cytoplasm or in the membrane. Third, in addition to activating expression of genes necessary for cooperative behaviors, detection of AIs results in activation of AI production (Novick et al. 1995; Seed et al. 1995). This

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feed-forward autoinduction loop presumably promotes synchrony in the population.

Gram-positive and Gram-negative bacteria use different types of QS systems (Fig. 1 shows the four paradigmatic QS wiring diagrams). Gram-positive bacteria use peptides, called autoinducing peptides (AIPs), as signaling molecules. Once produced in the cell, AIPs are processed and secreted. When the extracellular concentration of the AIP is high, which occurs at HCD, it binds to a cognate membrane-bound two-component histidine kinase receptor. Usually, binding activates the receptor's kinase activity, it autophosphorylates, and passes phosphate to a cognate cytoplasmic response regulator. The phosphorylated response regulator activates transcription of the genes in the QS regulon (Fig. 1A). In some cases of Gram-positive bacterial QS, AIPs are transported back into the cell cytoplasm where they interact with transcription factors to modulate the transcription factor's activity and, in turn, modulate gene expression changes (Fig. 1B).

Gram-negative bacteria communicate using small molecules as AIs. These are either acyl-homoserine lactones (AHLs) or other molecules whose production depends on *S*-adenosylmethionine (SAM) as a substrate (Wei et al. 2011). AIs are produced in the cell and freely diffuse across the inner and outer membranes. When the concentration of AIs is sufficiently high, which occurs at HCD, they bind cytoplasmic receptors that are transcription factors. The AI-bound receptors regulate expression of the genes in the QS regulon (Fig. 1C). In some cases of Gram-negative bacterial QS, AIs are detected by two-component histidine kinase receptors that function analogously to those described in the preceding paragraph for Gram-positive QS bacteria (Fig. 1D).

Dozens of clinically-relevant bacteria use QS to regulate the collective production of virulence factors. Here, we highlight the QS systems of four human pathogens that exemplify the diversity of QS systems. First, we outline how *Staphylococcus aureus* uses the paradigmatic Agr system to regulate adhesion and production of virulence factors. We also discuss the PlcR/PapR system that controls virulence factor production in the Gram-positive bacterium *Bacillus cereus.*

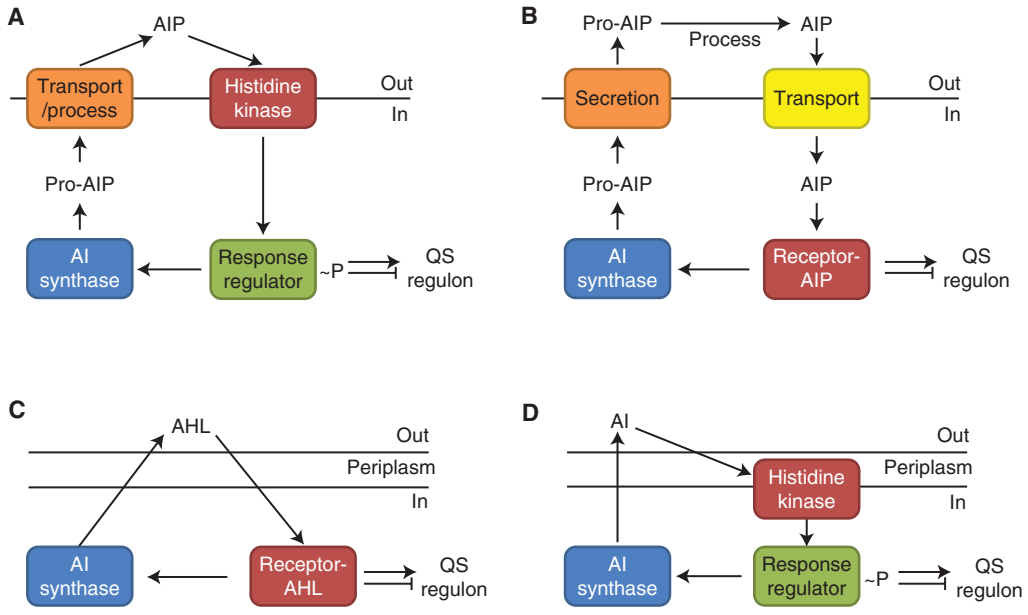


Figure 1. Canonical bacterial quorum-sensing (QS) circuits. Autoinducing peptide (AIP) QS in Gram-positive bacteria by (*A*) two-component signaling, or (*B*) an AIP-binding transcription factor. Small molecule QS in Gram-negative bacteria by (*C*) a LuxI/LuxR-type system, or (*D*) two-component signaling.

Next, we describe the canonical Gram-negative LuxI/LuxR QS circuit and how *Pseudomonas aeruginosa* exploits two such circuits arranged in tandem to control virulence factor production and biofilm formation. Finally, we discuss QS in *Vibrio cholerae*, a Gram-negative bacterium that uses two parallel two-component QS systems to control virulence factor production and biofilm formation. Importantly, in all of these cases, the QS circuits are tailored to promote the specific disease. *P. aeruginosa* and *S. aureus* cause persistent diseases while *V. cholerae* and *B. cereus* cause acute infections. We also outline efforts to develop inhibitors of these QS systems to be deployed as novel antimicrobials.

QS CONTROL OF VIRULENCE FACTORS IN GRAM-POSITIVE BACTERIA

Two-Component QS in Gram-Positive Bacteria

QS in Gram-positive bacteria relies on principles common to all QS circuits: production, detection, and response to AIs. In many Gram-positive bacteria, the AIs are oligopeptide AIPs that are detected by membrane-bound two-component signal transduction systems (Fig. 1A) (Havarstein et al. 1995; Ji et al. 1995; Solomon et al. 1996).

The AIPs are encoded as precursors (pro-AIPs) and are diverse in sequence and structure (Havarstein et al. 1995; Otto et al. 1998; Lazazzera 2001; Nakayama et al. 2001; Kalkum et al. 2003; Okada et al. 2005; Thoendel et al. 2011). Because the cell membrane is impermeable to peptides, specialized transporters are required to secrete AIPs. The AIP transporters also process the pro-AIPs. The final processed AIPs range in size from 5 to 17 amino acids, can be posttranslationally modified, and can be linear or cyclized (Magnuson et al. 1994; Havarstein et al. 1995; Mayville et al. 1999; Okada et al. 2005; Bouillaut et al. 2008). Extracellular AIPs are detected via membrane-bound two-component sensor kinases (Hoch and Silhavy 1995; Inouye and Dutta 2003; Simon et al. 2007). The sensor kinases autophosphorylate at conserved histidines when bound by the AIP. The phosphoryl group is passed from the histidine to a conserved aspartate on a cognate cytoplasmic response-regulator protein, and the phosphorylated response regulator controls expression of QS-target genes. In these Gram-positive QS circuits, the pro-AIP, transporter, histidine kinase receptor, and response regulator are typically encoded in an operon (Ji et al. 1995; Peterson et al. 2000). Expression of this operon is activated by the phosphorylated response regulator, resulting in an autoinducing feed-forward loop that synchronizes the QS response.

Some examples of Gram-positive QS behaviors are competence in *Streptococcus pneumonia* and *Bacillus subtilis* and sporulation in *B. subtilis* (Kleerebezem et al. 1997). QS controls virulence factor production in Gram-positive human pathogens including *S. aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Clostridium perfringens* (Autret et al. 2003; Podbielski and Kreikemeyer 2004; Ohtani et al. 2009; Riedel et al. 2009; Thoendel et al. 2011). The most well-studied system in this group of pathogens is the *S. aureus* Agr system (reviewed extensively in Thoendel et al. 2011).

S. aureus Quorum Sensing

S. aureus is found among the normal human skin flora. If the epithelial barrier is compromised, *S. aureus* can cause minor skin infections. These infections can lead to pneumonia, bacteremia, and sepsis (Lowy 1998; Massey et al. 2006). *S. aureus* is the leading cause of hospital-related infections in the United States. Its ability to cause disease depends on expression of an array of adhesion molecules, toxins, and compounds that affect the immune system. QS regulates expression of genes encoding these virulence factors.

S. aureus uses a canonical Gram-positive two-component QS system encoded by the *agr* locus (Fig. 2). The P2 promoter drives expression of a transcript (RNAII), which encodes the four components of the QS system (Novick et al. 1995). *agrD* encodes the pro-AIP, which is processed to the final AIP and secreted by the *trans*-membrane transporter protein AgrB (Ji et al.

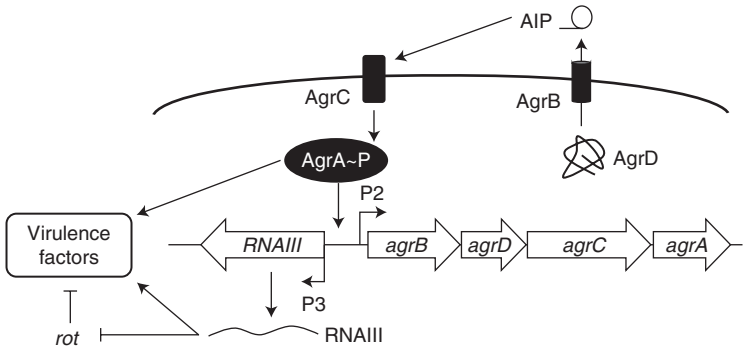


Figure 2. *S. aureus* Agr QS circuit. The autoinducing peptide (AIP) is synthesized as a precursor from *agrD*. The AIP transporter AgrB processes the precursor to the mature AIP and transports it out of the cell. AIPs are detected by a two-component signal transduction pathway. AgrC is the membrane-bound histidine kinase and AgrA is the response regulator. Phosphorylated AgrA activates the P2 and P3 promoters encoding the *agr* operon (called RNAII) and the RNAIII regulatory RNA, respectively. RNAIII posttranscriptionally activates virulence factor production and represses expression of *rot*, the repressor of toxins, leading to further derepression of virulence factors.

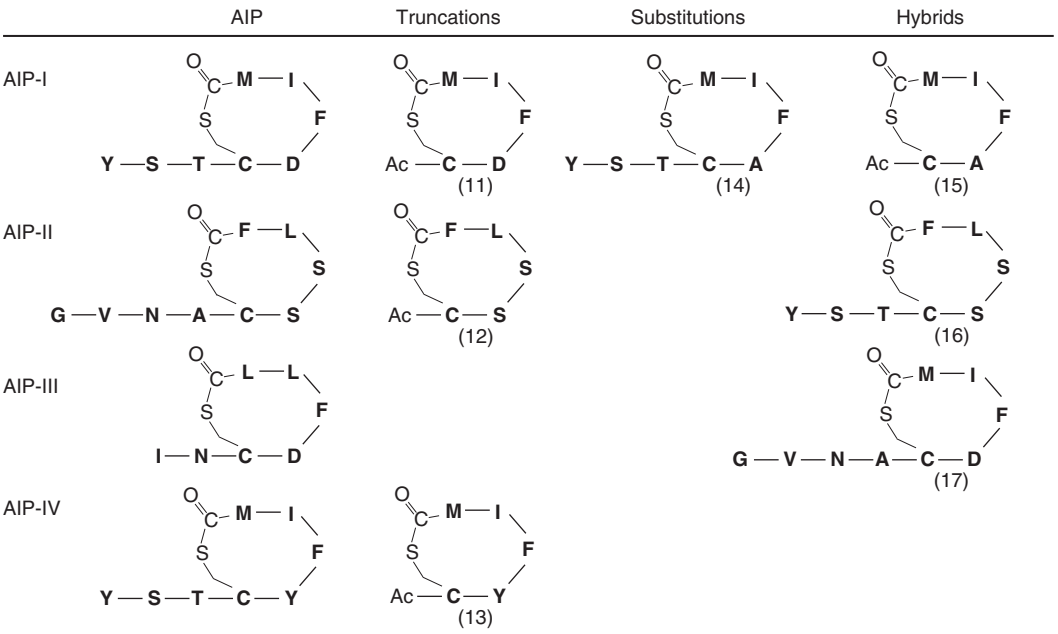
1995; Saenz et al. 2000; Thoendel and Horswill 2009, 2010; Thoendel et al. 2011). Processing involves truncating the 45-47 residue pro-AIP to a 7-9 residues peptide, coupled with cyclization of a five-membered peptide ring via a thiolactone bond between a central cysteine residue and the carboxyl terminus (Table 1). When the AIP accumulates, it binds the membrane-bound histidine kinase AgrC, which autophosphorylates at a conserved histidine and transfers the phosphate group to an aspartate on the response regulator AgrA (Lina et al. 1998). Phosphorylated AgrA binds upstream of the P2 promoter to autoinduce the *agr* operon (Novick et al. 1995).

In addition to activating the P2 promoter, phosphorylated AgrA activates the divergently encoded P3 promoter. The P3 promoter controls expression of RNAIII (Novick et al. 1993). The 5′ region of RNAIII harbors the *hld* gene, which encodes the virulence factor δ-hemolysin (Janzon and Arvidson 1990). A more prominent role for RNAIII is as a regulatory RNA (Novick et al. 1993). RNAIII has the dual-function of activating production of α-toxin and repressing expression of *rot*, fibronectin binding proteins A and B, protein A, coagulase, and other surface proteins (Morfeldt et al. 1995; Dunman et al. 2001). Repression of *rot*, which encodes a *r*epressor *o*f *t*oxins, leads to de-repression of additional toxins, proteases, lipases, enterotoxins, superantigens, and urease (Said-Salim et al. 2003; Geisinger et al. 2006). The net result of this QS regulatory cascade is down-regulation of surface virulence factors (such as protein A), and up-regulation of secreted virulence factors (such as α-toxin). Most of the effects of QS on regulation of virulence in *S. aureus* are mediated through direct and indirect regulation by RNAIII, however, phosphorylated AgrA also directly activates at least two additional virulence genes encoding phenol-soluble modulines (Queck et al. 2008).

Another key component of the *S. aureus* virulence program is biofilm development. In *S. aureus*, the *agr* system inhibits biofilm formation (Vuong et al. 2000; Boles and Horswill 2008). One interpretation of this finding is that establishing a biofilm community at LCD allows *S. aureus* time to grow to HCD and, at that point, it is optimally poised to secrete virulence factors (Yarwood et al. 2004; Boles and Horswill 2008). To facilitate its dispersal, *S. aureus* terminates biofilm production and decreases surface proteins and adhesions at HCD. This behavior is analogous to the strategy used by *V. cholerae* (see below).

Numerous additional regulatory factors converge at the P2 and P3 promoters, which add

Table 1. *S. aureus* AIPs and inhibitors



Inhibitors 11, 13, 15, 16, and 17 are from (Lyon et al. 2002); inhibitor 12 is from (Lyon et al. 2000); and inhibitor 14 is from (McDowell et al. 2001).

layers of regulation to the *S. aureus* QS circuitry, presumably ensuring that the QS program is expressed under the most ideal situations. There is evidence for regulation by ArlRS, CcpA, CodY, Rsr, σ^B, SarA, SarR, SarT, SarU, SarX, SarZ, Rsr, and SrrA/SrrB (Cheung and Projan 1994; Schmidt et al. 2001; Yarwood et al. 2001; Manna and Cheung 2003, 2006a,b; Liang et al. 2005; Seidl et al. 2006; Majerczyk et al. 2008; Lauderdale et al. 2009; Tamber and Cheung 2009; Tamber et al. 2010). One of the interesting themes common to several of these accessory *agr* regulators is that they provide *S. aureus* the ability to respond to extracellular environmental signals, in addition to AIPs, using the QS circuitry. For example, following extracellular stress, the alternative sigma factor σ^B interacts with core RNAP to direct transcription of surface proteins and pigment production, and inhibit expression of secreted toxins and proteases. RNAIII levels also increase when the σ^B gene is deleted (Lauderdale et al. 2009). No consensus sequence for σ^B exists at the P2 or P3 promoters, so it is likely that σ^B regulates some as-yet-unknown regulator of *agr*. σ^B regulation presumably ensures that *S. aureus* does not undergo QS, which is energetically costly, under conditions when the bacteria must dedicate resources to alleviating stress. Another example involves CodY, which responds to isoleucine limitation in *S. aureus*. Deletion of *codY* increases RNAII and RNAIII expression (Majerczyk et al. 2008; Pohl et al. 2009). CodY binding sites are located in the *agrC* gene (Majerczyk et al. 2010). Thus, CodY allows *S. aureus* to delay its QS response until it exists in a nutrient-rich environment. Finally, the SrrA/SrrB two-component system, which regulates gene expression in response to anaerobic environments, also controls the P2 and P3 promoter region via SrrB binding (Yarwood et al. 2001). Overexpression of SrrA/SrrB decreases virulence, likely due, at least in part, to inhibition of *agr* expression. Again, this regulation is thought to ensure that QS is not undertaken in a less than ideal environment.

A final interesting aspect of the *S. aureus* QS system is cross-competition among AIP specificity types. Hypervariability exists in the *agrD*

gene and a portion of the *agrB* gene (Dufour et al. 2002). This variability leads to production of one of four different types of *S. aureus* AIPs depending on the strain (Table 1). The identity of the AIP allows typing of *S. aureus* into four specificity groups (I–IV) (Ji et al. 1997; Jarraud et al. 2000). A corresponding hypervariability exists in the portion of the *agrC* gene encoding the sensing domain of the AIP receptor (Dufour et al. 2002). Thus, each specific AIP is detected by a coevolved cognate AgrC sensor. The presence of a noncognate AIP results in inhibition of QS, highlighting the specific nature of the AIP-receptor interactions. Specificity is proposed to be determined sterically and binding of the incorrect AIP-type can lead to stabilization of an inhibitory confirmation of AgrC, thus halting cell–cell signaling (Mayville et al. 1999; Lyon et al. 2002; Geisinger et al. 2009). The biological consequence of this mechanism is that the first strain to establish its QS cascade in the host is the one that causes the infection (Fleming et al. 2006).

S. aureus QS as a Therapeutic Target

The prevalence of *S. aureus* infections coupled with the emergence of methicillin-resistant *S. aureus* (MRSA) highlight the importance of studying and controlling this pathogen (Brumfitt and Hamilton-Miller 1989). The rationale for targeting the *S. aureus* QS system is that *agr* mutants are defective in virulence. The principal target considered to date is the receptor AgrC. Because the AIPs naturally cross-inhibit, analyses of their variations coupled with synthetic chemistry have provided a mechanistic understanding of AIP antagonism. By varying the length (Table 2, peptides 11–13) or amino acid sequence (Table 2, peptides 14) of the AIP or by combining substitutions and truncations to make hybrid AIPs (Table 2, peptides 15–17), universal inhibitors capable of out-competing all four AIP types have been designed (Lyon et al. 2000, 2002; McDowell et al. 2001).

The AIPs themselves have also been targeted for inhibition by inactivation through interaction with specific antibodies. The AIPs are poor antigens, however, monoclonal antibodies against AIPs coupled to carriers have been raised and are promising (Park et al. 2007). For example, screening of a library of monoclonal antibodies identified one antibody that reduced RNAIII and toxin production. This antibody acts as a highly-specific QS quencher.

One of the concerns with targeting the *S. aureus* QS system is the trade-off between virulence factor production and biofilm formation (Otto 2004). Inhibition of *agr* causes *S. aureus* to become more adherent because of increased biofilm formation. This facet of the circuit can lead to persistent colonization by *S. aureus*, which is of utmost concern for medically-implanted devices. Although there are reports that deletion of *agr* does not enhance biofilm formation under all conditions (Shenkman et al. 2001), targeting *agr* will likely become one part of a combination therapy for combating *S. aureus* and MRSA.

QS by Peptide-Binding Transcription Factors in Gram-Positive Bacteria

Some Gram-positive bacteria use a QS system in which AIPs, following release, are imported back into the cell and detected by cytoplasmic transcription factors (Fig. 1B). In these systems, the pro-AIP is secreted and processed into the mature AIP by extracellular proteases. Following transport back into the cell, the AIP binds to and alters the activity of a transcription factor (Slamti and Lereclus 2002).

Some examples of Gram-positive systems in which AIPs are imported include sporulation, competence, and enzyme production in *B. subtilis* (Pottathil and Lazazzera 2003) and plasmid transfer in *E. faecalis* (Dunny 2007). Additionally, AIP-bound transcription factors control virulence factor production in the *Bacillus cereus* group (Slamti and Lereclus 2002; Bouillaut et al. 2008) and we describe this system here (see review by Rocha-Estrada et al. 2010).

B. cereus Quorum Sensing

The *B. cereus* group of Gram-positive bacteria consists of several closely-related bacteria relevant to human health including *B. cereus*, *B.*

anthrasis, and *B. thuringiensis*. *B. cereus* causes both intestinal and nonintestinal infections in humans and is most commonly associated with food poisoning (Bottone 2010). Its ability to cause acute diarrheal disease is caused by the production and secretion of a variety of hemolysins, phospholipases, and toxins (Bottone 2010).

QS in *B. cereus* requires the transcription factor PlcR, which controls expression of most *B. cereus* virulence factors following binding to the intracellular AIP derived from the PapR protein (Fig. 3) (Slamti and Lereclus 2002). *papR* is encoded 70 basepairs downstream of *plcR*. PapR is 48 amino acids long and contains an amino-terminal signal peptide that targets it for the secretory pathway (Okstad et al. 1999). Once outside the cell, the PapR pro-AIP is processed by the secreted neutral protease B (NprB) to form the active AIP (Pomerantsev et al. 2009). *nprB* is encoded divergently from *plcR* and *nprB* expression is activated by AIP-bound PlcR (Okstad et al. 1999). NprB cleaves the pro-AIP PapR into peptides of 5, 7, 8, and 11 amino acids in length, all of which are derived from the carboxyl terminus of full-length PapR (Pomerantsev et al. 2009). Only the pentapeptide and heptapeptide activate PlcR activity, however, the heptapeptide causes maximal activation and is more prevalent in vivo (Bouillaut et al. 2008; Pomerantsev et al. 2009).

Similar to the *S. aureus* Agr system (see above), there is sequence diversity in the PapR AIPs that enables classification of members of the *B. cereus* group into four pherotypes. Specificity for the PlcR receptor stems from the identities of the first and fifth amino acids in the pentapetides: LPFE(F/Y), VP(F/Y)E(F/Y), MPFEF, and LPFEH (Slamti and Lereclus 2005). There is low cross-reactivity with heterologous PlcRs, indicating that the AIP and its receptor (PlcR) coevolved (Slamti and Lereclus 2005). Another interesting aspect of the pherotypes is that some contain different species, and can therefore communicate across species, whereas in other cases, different isolates of the same species fall into different subgroups and are prevented from communicating with one another (Slamti and Lereclus 2005; Rocha-Estrada et al. 2010).

The processed PapR AIP is imported back into the cell by the oligopeptide permease system (Opp) (Fig. 3) (Gominet et al. 2001). Once inside the cell, the AIP binds to the transcription factor PlcR, and this causes conformational changes in the DNA-binding domain of PlcR, facilitates PlcR oligomerization, DNA binding, and regulation of transcription (Declerck et al. 2007).

When PlcR interacts with the PapR AIP and oligomerizes, it binds to "PlcR boxes" to regulate transcription of target genes (Agaisse et al. 1999). PlcR controls expression of 45 genes, many of which encode extracellular proteins including several enterotoxins, hemolysins, phos-

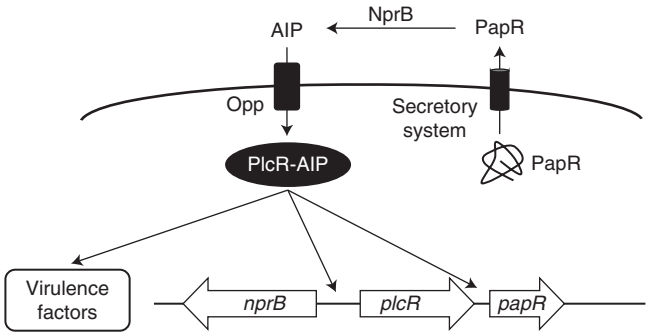


Figure 3. *B. cereus* QS circuit. The pro-AIP PapR is secreted and is then processed to the mature heptapeptide AIP by the extracellular protease NprB. The mature AIP is transported back into the cell by Opp. Intracellular AIP binds to the transcription factor PlcR and activates it. The PlcR–AIP complex regulates virulence factor production and activates expression of *papR*.

pholipases, and proteases (Lereclus et al. 1996; Gohar et al. 2002, 2008). As is the case in all QS systems, AIP-bound PlcR also feedback activates expression of *papR* (Lereclus et al. 1996). In addition to its role in regulating extracellular virulence factors, AIP-bound PlcR has other regulatory targets. These include two-component systems, transport systems, and GGDEF-containing proteins (Gohar et al. 2008). Finally, deletion of *plcR* results in increased biofilm production (Hsueh et al. 2006). However, despite its important role in regulating virulence factor production, disruption of *plcR* does not fully eliminate virulence (Lereclus et al. 2000; Callegan et al. 2003) because several additional systems feed into the *B. cereus* QS circuit and virulence factor regulation. These additional sensory inputs include sporulation through SpoOA $\sim$ P, nutritional state through CodY, motility through FlhA, and other two-component systems (Lereclus et al. 2000; Bouillaut et al. 2005; Brillard et al. 2008; Frenzel et al. 2012).

To date, no anti-PlcR/PapR QS molecules or screens for inhibitors of QS in *B. cereus* have been reported. Analogous to the *S. aureus* anti-QS strategies described above, the *B. cereus* AIPs could certainly be investigated for development into QS inhibitors. The NprB protease, the Opp transporter, and the PlcR receptor are other obvious candidates for exploration of whether or not they are vulnerable to small molecule inhibition.

QS CONTROL OF VIRULENCE IN GRAM-NEGATIVE BACTERIA

LuxI/LuxR Quorum Sensing

Gram-negative bacteria typically use LuxI/LuxR-type QS systems homologous to the first described QS system from the bioluminescent marine symbiotic bacterium *Vibrio fischeri* (Fig. 1C) (Ruby 1996; Hastings and Greenberg 1999). In these systems, the LuxI homolog is an AI synthase that catalyzes a reaction between SAM and an acyl carrier protein (ACP) to produce a freely diffusible acyl homoserine lactone (AHL) AI (Engebrecht and Silverman 1984; More et al. 1996; Schaefer et al. 1996; Ng and Bassler 2009). At high concentrations, AHL AIs bind to cognate cytoplasmic LuxR-like transcription factors. When not bound by AI, LuxR-type proteins are rapidly degraded, presumably to prevent bacteria from "short-circuiting" their QS systems. AI binding stabilizes the LuxR-type proteins, allowing them to fold, bind DNA, and activate transcription of target genes (Engebrecht et al. 1983; Engebrecht and Silverman 1984; Stevens et al. 1994; Zhu and Winans 1999, 2001). Typically, AHL-bound LuxR-type proteins also activate *luxI* expression, forming a feed-forward autoinduction loop that floods the vicinity with AI (Engebrecht et al. 1983; Fuqua and Winans 1994; Seed et al. 1995).

LuxI/LuxR homologs have been identified in more than 100 Gram-negative bacterial species (Case et al. 2008). AHLs produced by different bacteria possess different length side chains and side-chain decorations. Acyl chains ranging from C4 to C18 have been identified with modifications such as carbonyl and hydroxy moieties at the C3 position (Fuqua et al. 2001; Ng and Bassler 2009). This chemical diversity promotes intraspecies-specific bacterial cell–cell communication in that interactions with partner LuxR proteins are usually highly specific. The substrate binding pockets of the various LuxI homologs have correspondingly different sizes and shapes, ensuring accommodation of only a particular acyl-ACP for synthesis of a particular AI (Watson et al. 2002; Gould et al. 2004). Likewise, the AI-detecting LuxR homologs possess unique binding pockets that specifically accommodate particular AHL ligands (Vannini et al. 2002; Zhang et al. 2002; Yao et al. 2006; Bottomley et al. 2007; Chen et al. 2011).

Numerous Gram-negative pathogens control virulence factor production using LuxI/LuxR-type QS circuits. Some examples are LasI/LasR and RhlI/RhlR in *P. aeruginosa*, SmaI/SmaR in *Serratia marcescens*, VjbR (an orphan LuxR homolog) in *Brucella melitensis*, and CviI/CviR in *Chromobacterium violaceum* (Passador et al. 1993; Brint and Ohman 1995; McClean et al. 1997; Thomson et al. 2000; Weeks et al. 2010). We describe the *P. aeruginosa* systems as the canonical example here (reviewed in detail in Williams and Camara 2009).

P. aeruginosa QS

P. aeruginosa is a ubiquitous Gram-negative bacterium that can cause both acute and chronic infections in humans (Chugani and Greenberg 2007; Mena and Gerba 2009). *P. aeruginosa* infection generally depends on the host having a compromised immune system. Typically, *P. aeruginosa* infections are found in the lungs of people with cystic fibrosis (Lyczak et al. 2002; Zemanick et al. 2011). This infection leads to declined pulmonary function and increased mortality. *P. aeruginosa* also causes acute infections in people with compromised epithelial barriers; a common trait among patients with severe burns or tracheal intubation and mechanical ventilation (Bielecki et al. 2008). In both scenarios, *P. aeruginosa* uses QS to collectively produce a suite of virulence factors that contribute to its disease-causing ability.

P. aeruginosa harbors three QS systems: two LuxI/LuxR-type QS circuits that function in series to control expression of virulence factors as well as a third, non-LuxI/LuxR-type system called the *Pseudomonas* quinolone signal (PQS) system (Fig. 4). In the first circuit, the LuxI homolog LasI synthesizes 3-oxo-C12-homoserine lactone (3OC12HSL) (Table 2) (More et al. 1996; Val and Cronan 1998; Parsek et al. 1999; Gould et al. 2004; Bottomley et al. 2007). At HCD, this AI is detected by the cytoplasmic LuxR homolog LasR. The LasR–3OC12HSL complex activates transcription of target genes including those encoding virulence factors such as elastase, proteases, and exotoxin A (Gambello and Iglewski 1991; Gambello et al. 1993; Schuster et al. 2003, 2004). One of the LasR–3OC12HSL targets is *lasI*, which establishes an autoinducing feed-forward loop (Seed et al. 1995). Another target of regulation by LasR–3OC12HSL is a second *luxI* homolog called *rhlI* (Latifi et al. 1996; Pesci et al. 1997). RhlI synthesizes a second AI, butanoyl homoserine lactone (C4HSL) (Table 2) (Ochsner et al. 1994; Pearson et al. 1995). At high concentrations, this AI binds to RhlR, a second LuxR homolog. RhlR–C4HSL activates target genes, including those encoding elastase, proteases, pyocyanin, and siderophores (Schuster et al. 2003; Schuster and Greenberg 2007). Among

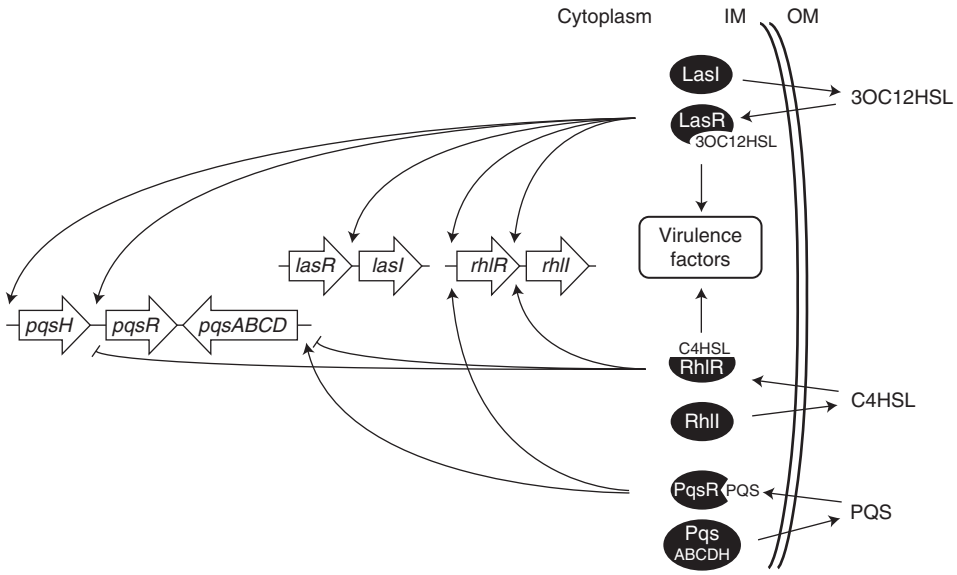
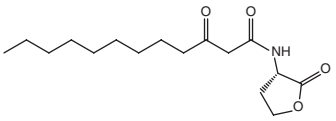
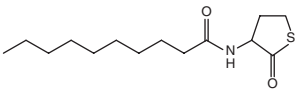
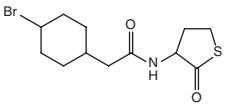
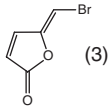
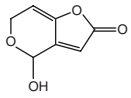
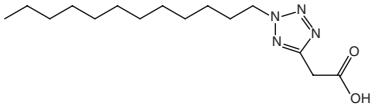
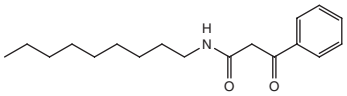
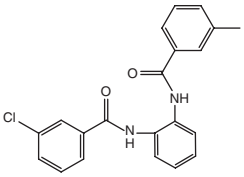
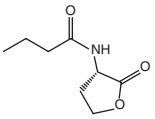
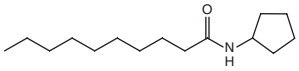
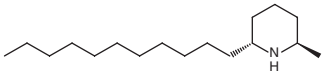
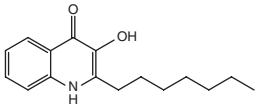
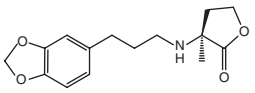


Figure 4. *P. aeruginosa* QS circuits. The three AI sythases, LasI, RhlI, and PqsABCDH, produce the AIs 3OC12HSL, C4HSL, and PQS, respectively. The AIs are detected by the cytoplasmic transcription factors LasR, RhlR, and PqsR, respectively. Each transcription factor regulates expression of its corresponding AI synthase as well as additional targets as indicated by the arrows.

Table 2. *P. aeruginosa* AIs and inhibitors

Receptor/target	Autoinducer	Antagonists	
LasR			(1)
			(2)
		 (3) 	(4)
			(5)
			(6)
			(7)
RhlR			(8)
			(9)
PqsR			
QscR			(10)

The three AIs (and their cognate receptors) are 3OC12HSL (LasR), C4HSL (RhlR), and PQS (PqsR). Inhibitors 1 and 2 are modified from 3OC12HSL by McInnis and Blackwell (2011); inhibitor 3 is a furanone described in Hentzer et al. (2002) and Wu et al. (2004); inhibitor 4 is patulin described in Rasmussen et al. (2005); inhibitors 5 and 6 are from a chemical library described in Muh et al. (2006b); inhibitor 7 is a triphenyl derivative from Muh et al. (2006a); inhibitor 8 is C10-acyl-cyclopentylamine from Ishida et al. (2007); inhibitor 9 is solenopsin from Park et al. (2008); and compound 10 is propanoyl homoserine lactone from Mattmann et al. (2008).

its targets is *rhlI*, leading to autoinduction of this second QS circuit.

The crystal structure of the ligand binding domain of LasR bound to 3OC12HSL has been solved, revealing interactions between the receptor protein and the AI (Bottomley et al. 2007). Structures of other LuxR-type QS regulators, TraR, SdiR, and CviR, have also been solved enabling comparative analysis (Vannini et al. 2002; Zhang et al. 2002; Yao et al. 2006; Chen et al. 2011). In all cases, the common lactone head group of each AHL is bound by conserved residues in the binding pocket, whereas the residues forming the pockets for the different acyl tails are diverse. Comparing the LasR binding pocket for 3OC12HSL with the TraR binding pocket for 3OC8HSL shows different sizes and hydrophobic characteristics in the channels that accommodate the different acyl chains. These structures coupled with biochemical data indicate that binding of ligand to the receptor stabilizes folding of the hydrophobic core of the protein. Once folded properly, LasR (and presumably other LuxR-type receptors) can dimerize, bind DNA, and activate transcription (Zhu and Winans 2001; Kiratisin et al. 2002; Schuster et al. 2004; Urbanowski et al. 2004).

P. aeruginosa uses an additional non-LuxI/LuxR QS system to control virulence factor gene expression. PQS, 2-heptyl-3-hydroxi-4-quinolone, is produced by PqsA, PqsB, PqsC, PqsD, and PqsH and is detected by the regulator PqsR (also called MvfR) (Fig. 4 and Table 2). Expression of *pqsH* and *pqsR* is activated by LasR–3OC12HSL, whereas RhlR–C4HSL represses *pqsABCD* and *pqsR* (Gallagher et al. 2002; Deziel et al. 2004; Xiao et al. 2006b). PqsR–PQS autoinduces PQS synthesis and further activates *rhlI* and *rhlR* expression (Xiao et al. 2006a; Diggle et al. 2007). Thus, the PQS circuit is intimately tied to the LasI/LasR and RhlI/RhlR QS systems and, therefore, also influences virulence factor production.

P. aeruginosa QS-activated virulence factors include elastase, proteases, pyocyanin, lectin, swarming motility, rhamnolipids, and toxins. Although distinct regulons for LasR–3OC12HSL and RhlR–C4HSL have been reported, it is apparent that there is extensive overlap between the regulons (Whiteley et al. 1999; Schuster et al. 2003, 2004; Wagner et al. 2004; Schuster and Greenberg 2007). Thus, most genes originally thought to be targeted only by LasR or only by RhlR can in fact be activated by either one. For example, a Δ*lasR* mutant, which is defective for *rhlI* induction, expresses virulence factors originally reported to be LasR-dependent (Dekimpe and Deziel 2009). Apparently, low level *rhlI* and *rhlR* expression promotes the eventual accumulation of C4HSL and autoinduction of the RhlI/RhlR system. Accordingly, activation of virulence factors is delayed in the Δ*lasR* strain. These findings are of medical relevance because many clinical isolates of *P. aeruginosa* possess mutations in *lasR* (Smith et al. 2006).

Another QS-controlled activity in *P. aeruginosa* is biofilm formation. Although regulation of biofilm formation in *P. aeruginosa* largely depends on additional environmental signals, QS regulation of rhamnolipids, swarming motility, and siderophores also contribute to *P. aeruginosa* biofilm formation (Ochsner et al. 1994; Deziel et al. 2003; De Kievit 2008; Patriquin et al. 2008; Rahman et al. 2010).

Up to 10% of the *P. aeruginosa* genome is controlled by QS (Schuster and Greenberg 2006), thus it is not surprising that there are additional levels of regulation impinging on the QS circuits. Vfr, AlgR, PhoR/B, RpoN, RpoS, DksA, RelA, GidA, QscR, RsmY, RsmZ, RsmA, PrrF, PhrS, VqsM, VqsR, and RsaL have all been reported to feed information into the *P. aeruginosa* QS circuit (Albus et al. 1997; de Kievit et al. 1999; Branny et al. 2001; Pessi et al. 2001; van Delden et al. 2001; Heurlier et al. 2003; Ledgham et al. 2003; Medina et al. 2003; Hogardt et al. 2004; Juhas et al. 2004; Dong et al. 2005; Jensen et al. 2006; Morici et al. 2007; Schuster and Greenberg 2007; Gupta et al. 2009; Sonnleitner et al. 2010). These accessory regulators of *P. aeruginosa* QS presumably fine-tune the network so that virulence factor production occurs with optimal precision. Indeed, when AIs are added to exponentially growing *P. aeruginosa*, they do not induce expression of the entire QS regulon (Schuster and Greenberg 2007). Only when cells are exposed to stimuli

associated with stationary phase as well as AIs is the complete set of QS targets launched. Although there are several fascinating regulatory events, here we highlight only two posttranscriptional regulation mechanisms.

QscR

QscR is an orphan LuxR homolog that does not have a partner LuxI homolog. QscR can, however, bind to the AI produced by LasI, 3OC12HSL (Lequette et al. 2006; Lintz et al. 2011; Oinuma and Greenberg 2011). Additionally, it forms mixed dimers with LasR and RhlR, rendering them inactive (Ledgham et al. 2003). Thus, QscR likely prevents aberrant QS responses before the cells reaching "a quorum."

sRNAs

Small regulatory RNAs (sRNAs) in bacteria act by basepairing with mRNA targets to repress or activate gene expression (reviewed in Gottesman 2004; Papenfort and Vogel 2010; Storz et al. 2011). In *P. aeruginosa*, the sRNAs PrrF1, PrrF2, and PhrS regulate the PQS system in response to limiting iron (PrrF1 and PrrF2) or limiting oxygen (PhrS) (Wilderman et al. 2004; Sonnleitner et al. 2010; Sonnleitner and Haas 2011). PhrS stimulates translation of an open reading frame in the 5′ end of the *pqsR* mRNA and this action activates *pqsR* translation by altering the secondary structure surrounding the *pqrR* RBS (Sonnleitner et al. 2010). PrrF1 and PrrF2 repress expression of genes encoding enzymes that degrade a precursor of PQS (anthranilate), thus allowing increased PQS under low iron conditions (Oglesby et al. 2008; Oglesby-Sherrouse and Vasil 2010; Sonnleitner and Haas 2011). Two other sRNAs involved in *P. aeruginosa* QS regulation are RsmY and RsmZ, sRNAs that are activated by the GacS/GacA two-component system in response to limiting magnesium and other unknown signals (Kay et al. 2006; Mulcahy and Lewenza 2011; O'Callaghan et al. 2011). RsmY and RsmZ repress the transcription factor RsmA, which, in turn, represses production of both 3OC12HSL and C4HSL (Pessi et al. 2001; Kay et al. 2006).

P. aeruginosa QS as a Therapeutic Target

The *P. aeruginosa* QS circuits make attractive targets for novel antimicrobials because QS controls virulence factor production and no homologs to known QS components exist in humans. This is especially critical in the treatment of persistent infections in cystic fibrosis patients given the resistance of many *P. aeruginosa* isolates to available antibiotics. Small molecule inhibitors of *P. aeruginosa* QS have been extensively reviewed (Mattmann and Blackwell 2010). Here we highlight a few recent advances.

Because LasR sits at the top of the *P. aeruginosa* QS cascade, identifying LasR inhibitors has been a major focus. Competitive inhibitors have been reported that contain modifications to the native 3OC12HSL ligand (Geske et al. 2005, 2007, 2008; Mattmann and Blackwell 2010; McInnis and Blackwell 2011). In this realm, alterations to both the head group and the acyl tail have led to molecules that out-compete 3OC12HSL for binding to LasR (Table 2, compounds 1 and 2). Importantly, some of these modifications, such as substitution of the lactone ring for a thiolactone ring (McInnis and Blackwell 2011), are useful because of their increased stability under biological conditions. Second, natural products have been isolated that inhibit QS by antagonizing LasR (Hentzer et al. 2002; Wu et al. 2004; Rasmussen et al. 2005; Ren et al. 2005; Kim et al. 2008). These inhibitors include furanones and patulin (Table 2, compounds 3 and 4, respectively), which have been further modified to increase their efficacy. Finally, high-throughput screens of small molecule libraries have revealed additional scaffolds for the design of inhibitors (Muh et al. 2006a,b; Borlee et al. 2010) (for example, Table 2, compounds 5–7). A potential complication in targeting LasR is that, as mentioned, some clinical *P. aeruginosa* isolates possess defective LasR proteins (Smith et al. 2006). Nonetheless, establishment of the initial infection is known to be LasI/LasR-dependent, suggesting that, at a minimum, LasR inhibitors could be used as prophylactics.

Other factors involved in *P. aeruginosa* QS have also been targeted for drug discovery. For

example, inhibitors of LasR have been successfully modified to act as competitive inhibitors of RhlR and in some cases these molecules have proven to be potent inhibitors of both LasR and RhlR (Table 2, compound 8) (Smith et al. 2003; Glansdorp et al. 2004; Ishida et al. 2007). Natural products have been isolated that inhibit RhlR (Table 2, compound 9) (Park et al. 2008).

A final approach is to target regulators that affect both the LasI/LasR and RhlI/RhlR QS systems. For example, a small molecule library was screened for agonists and antagonists of QscR, which, as described, influences both the LasI/LasR and RhlI/RhlR systems (Lee et al. 2006; Mattmann et al. 2008, 2011; Amara et al. 2009; Liu et al. 2010). By agonizing QscR, it could be possible to diminish the overall QS response and thus prevent or delay expression of virulence factors. Interestingly, some non-natural AHLs that target QscR also inhibit LasR, raising the intriguing possibility of a compound that can act broadly to target all of the *P. aeruginosa* QS systems (Table 2, compound 10) (Mattmann and Blackwell 2010).

V. cholerae Quorum Sensing

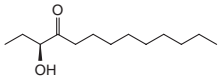
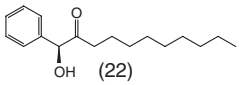
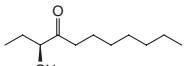
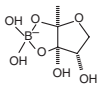
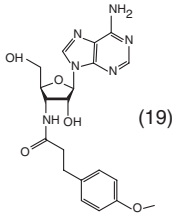
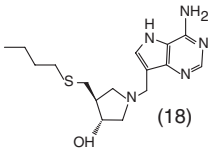
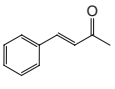
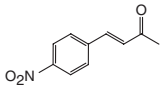
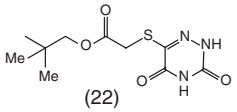
V. cholerae is the causative agent of the disease cholera (Faruque et al. 1998). This diarrheal disease is endemic in underdeveloped regions and has epidemic and pandemic potential, especially when clean water supplies are compromised. The principal symptom of the disease, profuse watery diarrhea, can lead to dehydration and death if not properly treated. This symptom is caused by an enterotoxin called cholera toxin, expression of which is controlled by QS (Jobling and Holmes 1997; Zhu et al. 2002).

V. cholerae, a Gram-negative bacterium, produces and responds to two AIs using two parallel QS circuits. One AI is (S)-3-hyroxytridecan-4-one (CAI-1), which is synthesized by the CqsA enzyme using SAM and decanoyl-coenzyme A as substrates (Table 3) (Higgins et al. 2007; Ng et al. 2011; Wei et al. 2011). Homologs of CqsA have been identified in all vibrio species consistent with a proposed role for CAI-1 as an intergenus communication molecule, allowing communication among other vibrios harboring *cqsA* (Miller et al. 2002; Henke and Bassler 2004; Higgins et al. 2007). The second AI, AI-2, is synthesized by LuxS. LuxS converts the SAM cycle intermediate *S*-ribosylhomocysteine to 4,5-dihydroxy-2,3-penanedionine (DPD) and homocysteine. DPD spontaneously converts into AI-2 (Table 3) (Surette et al. 1999; Schauder et al. 2001; Chen et al. 2002). Homologs of *luxS* exist in hundreds of Gram-negative and Gram-positive bacteria, consistent with a role for AI-2 in interspecies communication that allows other *luxS*-encoding bacteria in a particular environment to contribute to the overall cell-density information (Federle and Bassler 2003; Xavier and Bassler 2005). Thus, using two different AIs presumably allows *V. cholerae* to detect both the number of other vibrios and the total number of bacteria in the environment.

V. cholerae detects CAI-1 and AI-2 using two parallel membrane-bound two-component receptors (Fig. 5). CqsS detects CAI-1 and LuxPQ detects AI-2 (Bassler et al. 1994; Neiditch et al. 2005, 2006; Ng et al. 2010, 2011). In the absence of ligand, at LCD, both of these receptors function as kinases. Upon autophosphorylation, CqsS and LuxPQ transfer phosphate to the shared response regulator LuxO via the phosphotransfer protein LuxU (Bassler et al. 1994; Freeman and Bassler 1999a,b; Lilley and Bassler 2000). Phosphorylated LuxO activates expression of genes encoding four homologous quorum-regulatory sRNAs, called Qrr1-4 (Lenz et al. 2004). Transcription of the *qrr* sRNA genes is directed by the alternative sigma factor σ^{54}. To date, the four *qrr* promoters and the *luxO* promoter itself are the only known targets for regulation by LuxO.

At LCD, the Qrr sRNAs are expressed and regulate target mRNAs by base-pairing. The principal targets of the Qrr RNAs are the mRNAs encoding the master QS regulators HapR and AphA (Lenz et al. 2004; Rutherford et al. 2011). However, the Qrr sRNAs have opposite effects on these two mRNA targets. The Qrr sRNAs base-pair with the *hapR* mRNA overlapping the RBS, prevent ribosome binding, and facilitate degradation of *hapR* mRNA (Lenz et al. 2004; Tu and Bassler 2007). The Qrr sRNAs activate production of *aphA* by binding to the

Table 3. *V. cholerae* AIs and inhibitors

Receptor/target	Autoinducer	Antagonists
CqsS		 (22)  (23)
LuxPQ		 (19)
MTAN		 (18)
HapR		 (20)  (21)
LuxO		 (22)

The autoinducers are CAI-1 and AI-2. Inhibitor 18 is a MTAN transition state analog from Schramm et al. (2008); inhibitor 19 is a modified nucleoside from Brackman et al. (2009); inhibitors 20 and 21 are cinnamaldehydes described in Niu et al. (2006); Brackman et al. (2008, 2011); compounds 22 (phenyl-CAI-1) and 23 (C8-CAI-1) are from Ng et al. (2010); and compound 24 a 6-thio-5-azauracil derivative from Ng et al. (2012).

untranslated region of the *aphA* mRNA and inducing an alternative secondary structure, which reveals the RBS to allow translation (Rutherford et al. 2011; Shao and Bassler 2012). Thus, at LCD, the Qrr sRNAs prevent *hapR* expression and activate *aphA* expression so that HapR levels are low and AphA levels are high.

When CAI-1 and AI-2 accumulate at HCD, they bind CqsS and LuxPQ, respectively, which switches the receptors to phosphatases (Neiditch et al. 2006; Ng et al. 2010). It is not known how AI-2 impinges on in the LuxPQ kinase/phosphatase reaction but CAI-1 functions by inhibiting CqsS autophosphorylation (Wei et al. 2012). CAI-1 has no effect on the phosphotransfer step or the CqsS phosphatase activity (Wei et al. 2012). Once bound by AIs, LuxPQ and CqsS de-phosphorylate LuxO, leading to cessation of *qrr* expression, and the freeing of the *hapR* mRNA for translation. In the absence of Qrr sRNAs, in contrast, the *aphA* mRNA assumes a conformation refractory to

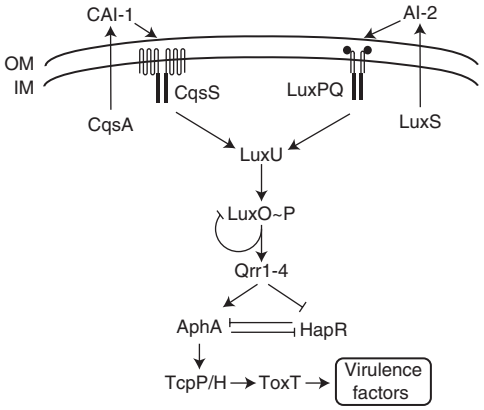


Figure 5. *V. cholerae* QS circuit. *V. cholerae* detects CAI-1 (produced by CqsA) and AI-2 (produced by LuxS) via two two-component histidine kinases, CqsS and LuxPQ, respectively. In the absence of AIs at LCD, the receptors function as kinases resulting in phosphorylation of LuxO, via LuxU, and activation of *qrr* gene expression. The Qrr sRNAs activate *aphA* and repress *hapR* expression. AphA represses *hapR* and activates *tcpPH*. TcpPH activates *toxT* expression and ToxT activates expression of the major virulence factors. At HCD, AIs bind the receptors switching them to phosphatases resulting in dephosphorylation of LuxO, cessation of *qrr* gene expression, and increased *hapR* expression. HapR represses *aphA* to shut down virulence factor production.

ribosome binding. The two master QS regulators are thus produced in reciprocal gradients with AphA highest at LCD and HapR highest at HCD. This dichotomy is reinforced by mutual repression whereby AphA represses *hapR* expression and HapR represses *aphA* expression (Kovacikova and Skorupski 2002; Lin et al. 2007; Pompeani et al. 2008; Rutherford et al. 2011).

Interestingly, *V. cholerae* expresses its suite of virulence factors at LCD (Jobling and Holmes 1997; Zhu et al. 2002). At LCD, AphA, a winged-helix transcription factor, works together with a LysR-type transcription factor, AphB, to activate transcription of *tcpPH* (Kovacikova and Skorupski 1999, 2001; Skorupski and Taylor 1999). TcpP is a *trans*-membrane DNA binding protein, the function of which is enhanced by TcpH (Carroll et al. 1997; Beck et al. 2004; Matson et al. 2007). TcpPH activates expression of *toxT*. ToxT is a transcription factor that activates expression of the genes encoding cholera toxin and the toxin co-regulated pilus, which are the major *V. cholerae* virulence factors (Fig. 5) (Higgins et al. 1992; Withey and DiRita 2006; Matson et al. 2007). Expression of *toxT* is also activated by ToxRS, which is homologous to TcpPH (Peterson and Mekalanos 1988; Skorupski and Taylor 1997; Matson et al. 2007).

Along with other regulators, QS also controls biofilm formation in *V. cholerae* (Hammer and Bassler 2003; Zhu and Mekalanos 2003). Biofilm formation is activated at LCD and repressed at HCD. HapR, expressed at HCD, plays a major role in this aspect of the program. First, HapR directly represses genes encoding components of the biofilm factory (Yildiz et al. 2004). Second, HapR represses expression of two transcription factors that activate biofilm formation genes, VpsR and VpsT (Beyhan et al. 2007; Waters et al. 2008; Shikuma et al. 2009; Srivastava et al. 2011). Third, HapR represses expression of genes required for the synthesis of the second messenger cyclic-di-GMP (Lim et al. 2006, 2007; Waters et al. 2008). Cyclic-di-GMP is sensed through the transcription factor VpsT to activate biofilm formation (Krasteva et al. 2010). This LCD biofilm lifestyle allows *V. cholerae* to remain attached to host tissue when virulence factors are expressed. At HCD, QS repression of biofilms and virulence factor production facilitates dispersal of *V. cholerae* back into the environment. This strategy allows *V. cholerae* to maximally compete for nutrients in the host at LCD and maximize its ability to exit and spread to other hosts once it reaches HCD (Nadell and Bassler 2011). Because a high infective dose is required, the host serves as the incubator, and it appears that when the titer is high enough to ensure infection of a new host, the population is dispersed.

V. cholerae QS Components as Targets for Therapeutics

The *V. cholerae* QS circuit has several possible targets for the discovery or design of novel antimicrobial agents. Some interesting approaches have targeted pathways involved in the synthesis

of the AIs. In the SAM cycle, MTAN, which is found only in bacteria, is a hydrolase that turns over methylthioadenosine to maintain the homeostatic SAM pool. This step is critical for the synthesis of both CAI-1 and AI-2 (Schauder et al. 2001; Wei et al. 2011). A synthetic inhibitor of MTAN blocks QS in *V. cholerae* without affecting growth (Table 3, compound 18) (Schramm et al. 2008). This MTAN inhibitor blocks AI-2 production in enterohemorrhagic *Escherichia coli* as well. In a screen for nucleoside analogs able to disrupt AI-2 QS, another molecule was identified that disrupts AI-2-based QS without affecting growth in *V. harveyi*, a close relative of *V. cholerae* (Table 3, compound 19) (Brackman et al. 2009). This compound likely targets the signaling pathway for the AI-2 receptor, LuxPQ, which is present in numerous vibrio species including *V. cholerae* (Fig. 5).

Cinnamaldehyde is a natural product that inhibits QS in vibrios (Niu et al. 2006). Cinnamaldehyde and derivatives are proposed to target HapR and its homologs (Table 3, compounds 20 and 21), thus inhibiting virulence factor production and biofilm formation without dramatically affecting growth (Brackman et al. 2008, 2011). These compounds increase survival of model host nematodes infected with different vibrios leading to the interesting possibility that HapR can be regulated by a cell permeable ligand.

Several synthetic ligands of the receptor CqsS have been identified. Using these molecules has been instrumental in determining the mechanism of signal recognition and discrimination in *V. cholerae* QS. For example, CqsS does not respond to CAI-1 molecules with bulky head groups (Table 3, compound 22) or shortened tail groups (Table 3, compound 23) (Ng et al. 2010). Indeed, such molecules are CqsS antagonists. Owing to the fact that *V. cholerae* expresses virulence factors at LCD in the absence of AIs, CqsS represents an interesting target because compounds that prematurely trigger QS (i.e., potent CqsS agonists) could prevent virulence factor expression.

Finally, in addition to the receptors and the transcription factor HapR, intermediate components in the QS circuit are targets for therapeutics. For example, a high-throughput chemical screen led to the identification of a LuxO antagonist (Ng et al. 2012). The molecule and potent analogs are all 6-thio-5-azauracil derivatives (Table 3, compound 24, for example). This family of molecules uncompetitively bind the preformed LuxO–ATP complex and inhibit ATP hydrolysis, which prevents transcription activation. When inhibited, LuxO is not able to activate expression of the *qrr* sRNA genes, thus locking *V. cholerae* into HCD mode and preventing expression of virulence factors (Fig. 5). Because LuxO exists in all vibrio QS circuits, this family of molecules has broad spectrum antivirulence activity in marine vibiros (Ng et al. 2012).

CONCLUDING REMARKS

QS is a vital regulatory mechanism used by many bacteria to control collective traits that allow bacteria to exploit particular niches. For example, QS enables access of symbionts to nutrient-rich environments in hosts (Ruby 1996). Bacterial populations use QS to control biofilm formation, which provides members of the population superior access nutrients and thus enables them to out-compete non-biofilm-producing neighbors (Nadell and Bassler 2011). Finally, as discussed in the above examples, bacteria that make their living by exploiting eukaryotic hosts have coupled production of the virulence factors necessary for a pathogenic lifestyle to their ability to detect changes in cell population density.

Four trends emerge when considering the examples described above. First, it is clear that QS gene regulation is the result of regulatory networks layered onto the basic AI production and detection apparatuses. Because of the global genetic programs controlled by QS, these additional regulatory mechanisms likely ensure that altered gene expression occurs only under precisely-defined conditions. Alternatively, given that QS regulates global gene expression patterns, perhaps other regulatory systems have co-opted QS systems to control additional products in response to particular environmental conditions. Second, it is interesting that in the

cases described here, as well as in other cases, QS is linked to biofilm production. Although the mechanism and timing of production differ among these species, in each case, QS plays a role in dictating when this process occurs. Members of biofilm communities most certainly benefit from the ability to communicate and, likewise, bacterial communication is probably more robust when the cells are in the intimate associations provided by biofilms. Third, there appears to be a prominent role for regulation involving sRNAs. QS controls expression of hundreds of genes and a commitment to a lifestyle that could be detrimental to the individual. One of the general benefits of regulation by sRNAs is the rapidity with which regulation can occur because translation and protein folding are not required to produce the regulator. Thus, it is possible that QS systems rely heavily on sRNAs to quickly adjust global gene expression programs as bacteria transition between disparate niches. Finally, small molecules analogous to those involved in QS are increasingly becoming recognized as key modulators of pathogenic behaviors and important players in biofilm formation in bacteria. Thus, strategies aimed at manipulating small molecule control of bacterial behaviors are now viewed as especially promising. Presumably, therapies that affect bacterial behavior will not be as prone to resistance as are the targets of traditional antibiotics that result in outright killing of bacteria or inhibition of their growth. Thus, therapeutics that interfere with small molecule-controlled pathways could have longer functional shelf lives than second and third generation antibiotics.

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