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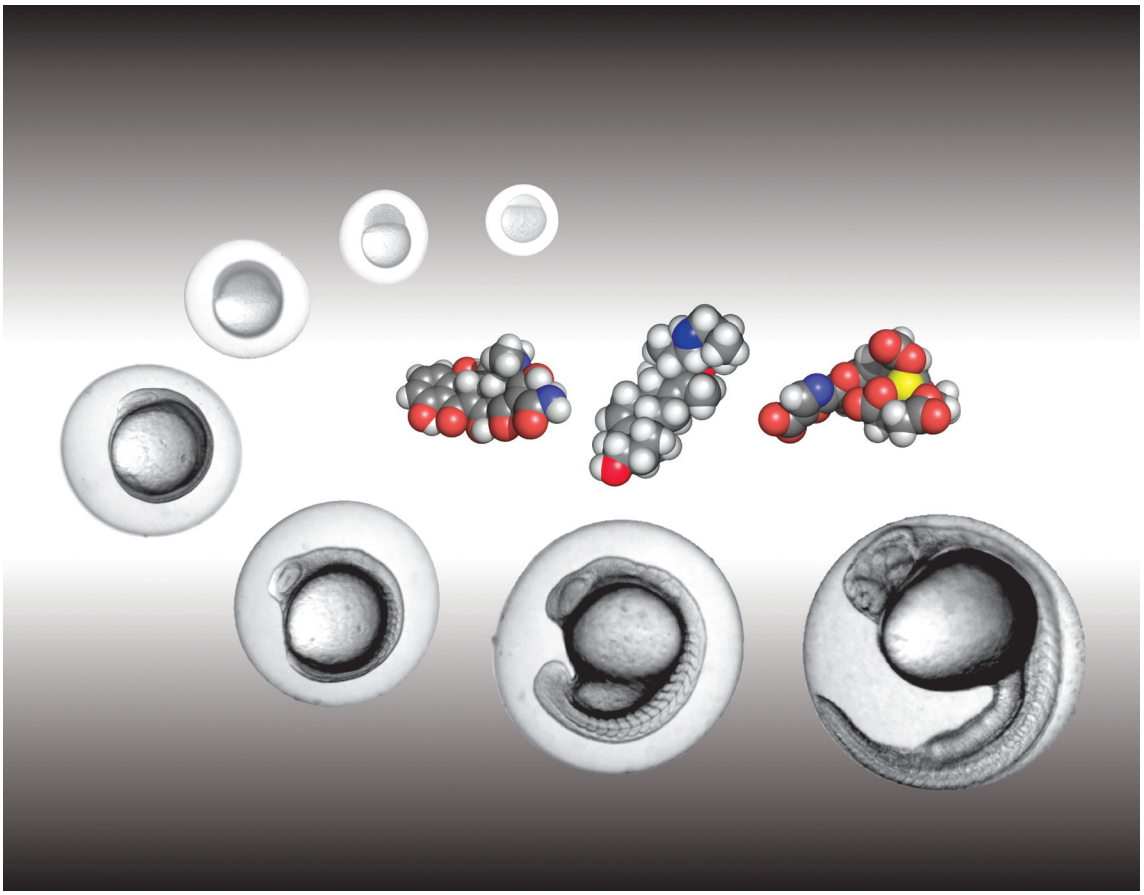

Interspecies and interkingdom communication mediated by bacterial quorum sensing†

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Quorum sensing (QS) has traditionally referred to a mechanism of communication within a species of bacteria. However, emerging research implicates QS in interspecies communication and competition, and such systems have been proposed in a wide variety of bacteria. This activity of bacterial QS also extends to relationships between bacteria and eukaryotes and host–pathogen interactions in both clinical and agricultural settings are of particular interest. These relationships are particularly pertinent in light of the rising prevalence of antibiotic resistant bacteria. In this *tutorial review* we describe bacterial QS and its capacity in interspecies and interkingdom interactions, as well as the corresponding eukaryotic responses.

Introduction

Under the cover of moonlight, the tiny Hawaiian bobtail squid (*Euprymna scolopes*) sneaks up on unsuspecting prey and avoids predation in the shallow coastal waters of Hawaii. Despite the paradox of moonlight as a camouflaging mechanism, the squid benefits from a symbiosis with the bioluminescent bacteria *Vibrio fischeri*. The bioluminescence produced by the bacteria allows the squid to blend in with the moonlight, so as to avoid casting shadows on the sea floor and alerting both predators and prey. However, the squid is not the only beneficiary in this relationship, as the bacteria gain a safe haven and easy access to nutrients. After a night of hunting and feeding, the amount of bacteria in the squid's light organ becomes too great for the squid to feed, and almost all of the bacteria are released into the ocean and bioluminescence subsides. During the day as the squid sleeps, the remaining bacteria multiply and bioluminescence is restored by nightfall.[1] This correlation between bacterial growth and light production was the subject of intense scrutiny, which ultimately led to the discovery of the regulation of bioluminescence through the secretion and detection of small molecules by the bacteria.[2,3]

Through the exchange of small chemical signals, bacteria monitor their population density and regulate gene expression in a population-dependent manner known as quorum sensing (QS). This process was initially referred to as ''autoinduction'', and consequently the small molecule signals were termed autoinducers.[4] Traditionally, autoinducers have been classified into two major groups: acyl homoserine lactones (AHLs), used by Gram-negative bacteria; and oligopeptides, used by Gram-positive bacteria (Fig. 1).[5,6] However, it is becoming increasingly evident that bacteria are not limited by these two classes of signals, as several different autoinducers have been identified in both Gram-positive and -negative bacteria, including the pseudomonas quinolone signal (PQS), bradyoxetin, and AI-2.[7]

Autoinducers accumulate in the environment as a function of bacterial population, and essentially, QS serves as a mechanism for bacteria to determine their population densities. Knowledge of their surroundings obtained through QS allows

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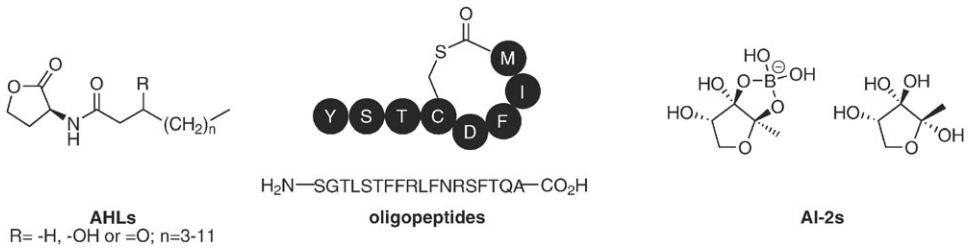

Fig. 1 General structures of major classes of autoinducers.

bacteria to regulate gene expression and perform specific functions beneficial only when carried out by a large number of cells. Recently, the requirement for cell density, or a "quorum" of cells, has been questioned in favor of a mechanism termed diffusion sensing, in which gene expression is under the control of the spatial organization of cells.[8] For the purposes of this review, we will use the term "quorum sensing" as a general term encompassing either density or diffusion sensing. Examples of QS-controlled processes include biofilm formation, virulence factor expression, and bioluminescence. Microbial communication and the resulting concerted gene expression allow bacterial communities to act as, and develop relationships with, multicellular organisms as in the case of *Euprymna* and *V. fischeri*. In a more nefarious example of QS providing for interactions with higher organisms, the Gram-negative bacteria *Pseudomonas aeruginosa* uses QS to regulate the formation of biofilms in its colonization of human hosts. These biofilms are often isolated from the lungs of cystic fibrosis (CF) patients, and, once formed, are often deadly for the patient.

The ecological roles of *V. fischeri* and *P. aeruginosa* are just two examples of QS-based interactions with multicellular organisms. The small molecule QS signals released by bacteria regulate interactions with other species of bacteria, as well as with other kingdoms, and mechanistically may arise through recognition of the signal by other cells whether they are prokaryotic or eukaryotic. Alternatively, these relationships may arise through the direct action of autoinducers on the target cells, exemplified by the antimicrobial activity of certain signals towards other species. Essential to the notion of interspecies and interkingdom signaling is the fact that signal transmission is not unidirectional, as certain ubiquitous QS signals have been proposed to be released and recognized by several different bacterial species. Interestingly, eukaryotes have also been found to respond to the signals of bacteria through production of their own small molecules. This review will cover these interspecies and interkingdom affairs involving the QS systems of bacteria, with a focus on the chemical signals used to mediate the relationships.

AI-2 based signaling

As described above, QS signals have traditionally been classified into two groups, AHLs and oligopeptides, that correspond to the Gram-classification of the bacteria that produce them. However, the AI-2 QS system has been proposed to exist in both Gram-positive and Gram-negative strains, and the synthase for 4,5-dihydroxy-2,3-pentanedione (DPD), the precursor to AI-2, has been found in over 70 bacterial species (Fig. 2). The first example of AI-2 based signaling was described by Bassler *et al.* during observations of QS activity in an AHL deficient strain of the Gram-negative bacteria *Vibrio harveyi*.[9] Despite the absence of the natural homoserine lactone autoinducer, the bacteria remained capable of producing bioluminescence, implying the presence of a second QS pathway. A previous study by Greenberg *et al.* in 1979 detailing the ability of cell free supernatants of nonluminous bacteria to induce production of bioluminescence in *V. harveyi* prompted the notion of the AI-2 system as a source of interspecies signaling.[10]

Later work by the Bassler group identified the gene for the AI-2 synthase in *V. harveyi*, *E. coli*, and *S. typhimurium*, termed *luxS*;[11] since its discovery this gene has been correlated to a variety of phenotypes including bioluminescence production,

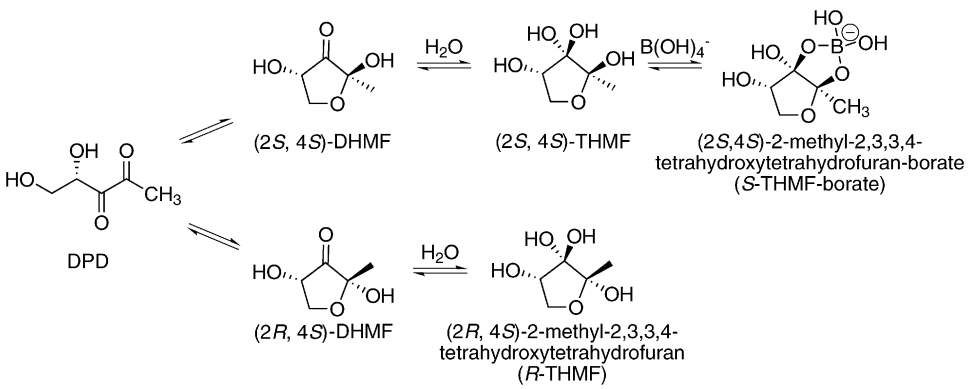

Fig. 2 Equilibrium of the biologically active forms of DPD. (*S*)-THMF-borate is the AI-2 signal used by *V. harveyi*, and (*R*)-THMF is the AI-2 signal used by *S. typhimurium*.

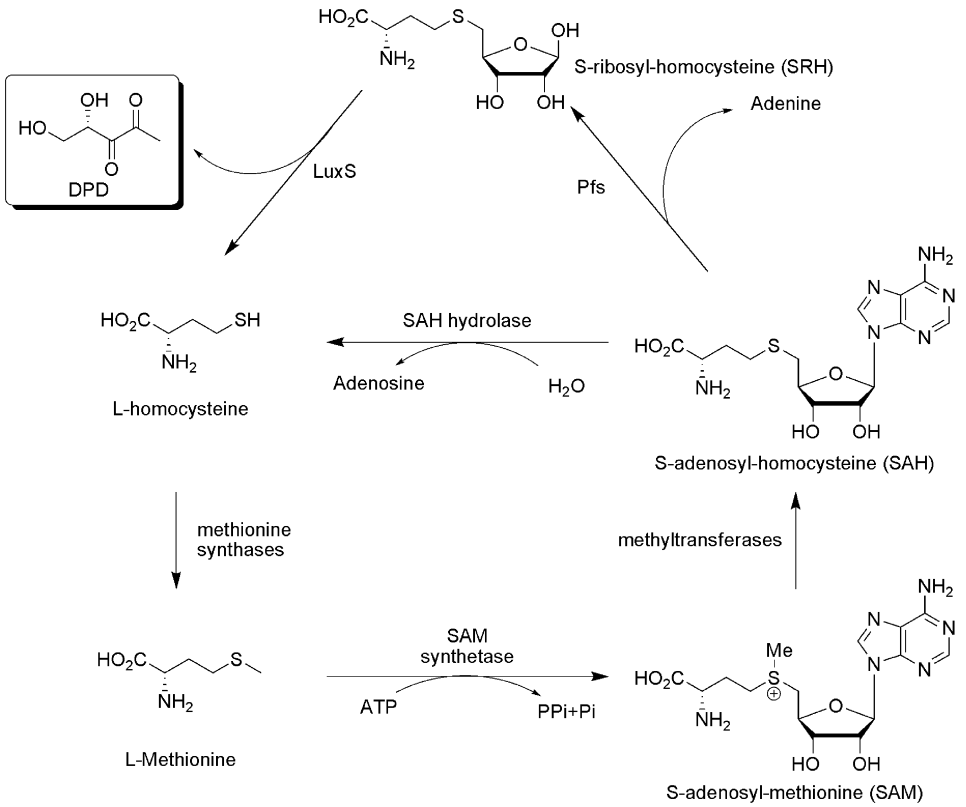

Fig. 3 The activated methyl cycle and its involvement in the biosynthesis of DPD.

expression of virulence factors, and biofilm formation. However, the primary method for determining AI-2 dependent phenotypes has been through the generation of *luxS* null mutants. This technique has been the target of criticism, as LuxS plays a role in the activated methyl cycle in many bacterial species (Fig. 3).[12] In this process, LuxS is involved in the removal of the toxic intermediate *S*-adenosylhomocysteine, which ultimately leads to the production of DPD. Due to the role of LuxS in this process, the observed phenotypes of *luxS* null mutants may be due not only to the inability to communicate *via* AI-2 but also changes in the metabolic fitness of the species in question. In spite of this criticism, several reports have provided evidence in support of AI-2 as an interspecies signal.

The first structure of AI-2 was solved, using X-ray crystallography, complexed with the *V. harveyi* sensor protein LuxP.[13] In this complex, AI-2 was found to exist as a furanosyl borate diester (*S*-THMF-borate, Fig. 2), a highly unusual structure as boron is not commonly associated with a functional role in biological systems. The structure of this AI-2 signal was thought to arise from the complexation of boronic acid by the cyclized form of DPD, the product of LuxS. Chemical confirmation of this biosynthetic pathway was later provided through chemical synthesis and biological evaluation of DPD by our laboratory.[14] Subsequently, the AI-2 signal in *S. typhimurium* was determined to be the furanosyl form of "R-DPD" without boron ((2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran, *R*-THMF), leading to the redefinition of AI-2 autoinducers as a group of signals derived from DPD.[15] This finding reinforced the hypothesis of AI-2 as an interspecies signal in that the two identified AI-2 structures are both formed from DPD, implying that DPD released by one species may be detected and recognized by several different species. Under this hypothesis, DPD is synthesized by LuxS, released by the cell and, due to the high reactivity of the 2,3-dicarbonyl motif, undergoes rapid intramolecular cyclization. This cyclization results in two stereoisomers at the quaternary carbon. Interestingly, *S. typhimurium* responds to the *2R* isomer, whereas *V. harveyi* recognizes a borate diester form of AI-2 derived from the 2*S* isomer (Fig. 2). The presence of boron in the structure of AI-2 was initially puzzling, but boronic acid has been shown to be present in sea water, the natural environment of *V. harveyi*. As such, the reactivity and variable stereochemistry of cyclic DPD highlights its potential for interspecies signaling and argues that modified DPD-based signals may be recognized in the extremely diverse environments inhabited by bacteria.

One example of interspecies communication *via* AI-2 is in the cooperative biofilm growth of two oral bacteria, *Actinomyces naeslundii* and *Streptococcus oralis*.[16] These bacteria represent two of the hundreds of bacteria present in oral plaque. Oral *Actinomyces* and *Streptococci* are two of the first bacteria to grow on freshly cleaned teeth, but do not grow apart from one another as single species biofilms. Rickard *et al.* have shown that the formation of healthy biofilms of *A. naeslundii* and *S. oralis* is dependent on the production of AI-2 by *S. oralis*. Biofilms grown with a *luxS* deletion mutant of *S. oralis* incapable of producing AI-2 exhibited 10-fold less biomass, but restoration with either genetic complementation or supplementation with synthetic AI-2 restored healthy biofilms. Although experiments were not performed to show the response of *A. naeslundii* to AI-2 itself, the

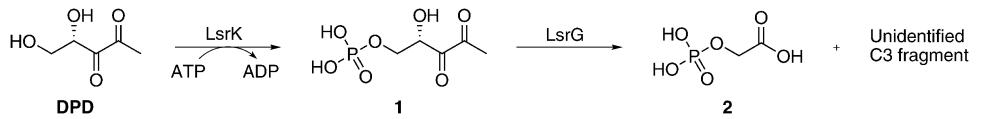

Fig. 4 Enzymatic degradation of DPD by the Lsr transporter system of *S. typhimurium*.

results from this study suggest a role for AI-2-based interspecies signaling in the formation of oral biofilms.

In a particularly elegant display of AI-2 recognition by multiple bacterial species, Xavier and Bassler have shown that some species of bacteria can manipulate the AI-2 signaling of other competing bacteria.[17] In this study, mixed cultures of *E. coli* and either *V. harveyi* or *V. cholera* were used to examine the effects exerted by each species on the QS of the other. For example, production or removal of the AI-2 signal from the culture by one bacteria resulted in the misjudgment of the cellular population by the other bacteria, resulting in premature or delayed QS activity. This study hinged on AI-2 release by *E. coli* in exponential phase, and the uptake and destruction of AI-2 by the Lsr (LuxS regulated) transporter system of *E. coli* at stationary phase. Thus, when wild type *E. coli* and *V. harveyi* are grown in co-culture, the bioluminescence and type III secretion of *V. harveyi*, two AI-2 regulated behaviors, are severely hindered. However, when incubated with a strain of *E. coli* incapable of AI-2 internalization, the QS activity of *V. harveyi* was restored. The AI-2 internalization mechanism of *E. coli* was also found to have a similar effect on *V. cholera*, a bacteria that likely encounters *E. coli* in its infection of the gastrointestinal tract of a human host, which may be inhabited by both commensal and pathogenic *E. coli*.

A similar mechanism of AI-2 signal internalization by the Lsr transporter system of *S. typhimurium* has recently been characterized.[18] In this system, *R*-THMF binds to the transmembrane receptor LsrB, where it is subsequently internalized and subjected to a series of enzymatic reactions, wherein DPD is phosphorylated at the ring open C5 position (Fig. 4). Interestingly, upon AI-2 internalization, transcription of the *lsr* locus is activated (*i.e.*, a positive feedback loop), resulting in rapid AI-2 uptake. It was previously hypothesized that a phosphorylated form of DPD binds and antagonizes the *lsr* repressor, allowing activation of the Lsr transport system. Using mass spectrometry, thin layer chromatography, and NMR with purified proteins, products of the first two enzymatic reactions after AI-2 internalization were identified. The final reaction yields 2-phosphoglycolic acid **2**, a phosphorylated cleavage product of DPD. This sequestration and destruction of DPD by the *lsr* system of *S. typhimurium* led to the hypothesis that *S. typhimurium* has evolved the ability to respond to competing bacteria through interference with their communication. It is interesting that *S. typhimurium*, as well as *E. coli*, have developed an AI-2 dependent QS system simply for the destruction of the AI-2 signal. As such, the *lsr* systems of *E. coli* and *S. typhimurium* represent two concrete examples of AI-2 QS systems exerting their effects on the signaling of other bacteria.

PQS signaling and effects

Another QS system believed to play a role in interspecies signaling is the 2-alkyl-4-quinolone (4Q) system, initially described in *P. aeruginosa*. This signaling system is interlinked with the AHL-regulated QS system, in that transcriptome analysis revealed a 55% overlap between the genes regulated by each, likely due to the fact that the synthesis of PQS is regulated by the AHL-regulated QS system.[19] Communication in *P. aeruginosa via* the 4Q system is based on the exchange of 2-heptyl-3-hydroxy-4-quinolone, or the *Pseudomonas* quinolone signal (PQS), as well as other related molecules (Fig. 5). It has been shown that PQS is produced during *P. aeruginosa* infection, and has been implicated in the expression of several virulence factors and biofilm formation. In spite of this evidence for PQS roles, the discrete genes regulated solely by PQS have yet to be determined.[20]

Biosynthesis of PQS is regulated by the *pqsABCDE* regulon, which encodes proteins responsible for the generation of 2-heptyl-4(1*H*)-quinolone (HHQ). HHQ is released from the cell and taken up by neighboring cells, where it is converted to PQS through the action of PqsH, and re-released to mediate intercellular communication. The biosynthetic pathway of PQS has raised speculation of possible interspecies signaling effects of PQS, as the synthetic steps regulated by *pqsA-E* use anthranilic acid as a starting material (Fig. 6). Anthranilic acid is a key intermediate in both the phenazine antibiotic pathway and the common tryptophan biosynthetic pathway. The involvement of anthranilate in both the tryptophan and PQS pathways has prompted the hypothesis of 4Q molecules as interspecies signals. This hypothesis is also supported by the discovery of homologs of *pqs* genes in several species of *Pseudomonas* and *Burkholderia* sp. Indeed, the *pqsA* gene from *Burkholderia pseudomallei* is capable of complementation of a *P. aeruginosa pqsA* mutant, and *B. pseudomallei* itself has been shown to produce HHQ.[20]

Aside from their signaling role in *P. aeruginosa* and their proposed effects on interspecies communication, 4Q signals have been shown to exert deleterious effects on other species through antibacterial action against Gram-positive bacteria. In fact, the first reports of 4Q molecules came in 1889, when cultures of *P. aeruginosa* were shown to inhibit growth of *Bacillus anthracis* (anthrax) in rabbits.[21] One particularly intriguing example of the interspecies interactions mediated by 4Q molecules has been reported by Hofmann *et al*.[22] In this study, it was found that the PQS analog 4-hydroxy-2-heptylquinoline-*N*-oxide (HQNO, Fig. 5), a substance produced by *P. aeruginosa* with activity against Gram-positive bacteria, inhibits the respiration of *Staphylococcus aureus*, a bacteria

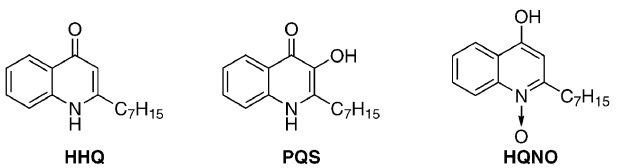

Fig. 5 Structures of various 4Q molecules.

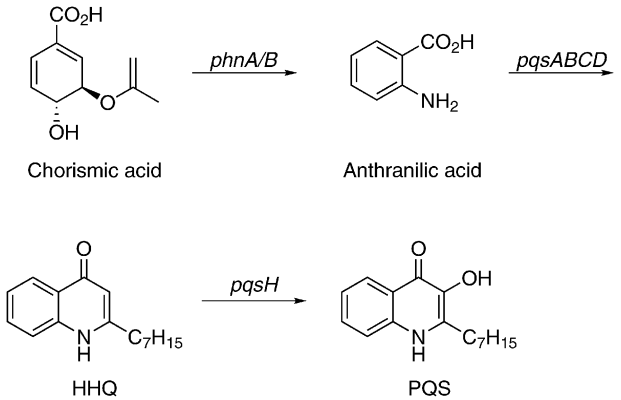

Fig. 6 Biosynthesis of HHQ and PQS from chorismic acid. The enzymes that catalyze the synthesis have not been characterized, but the genes encoding for PQS synthesis have been identified. Alternatively, anthranilic acid may be produced from the degradation of tryptophan (not shown).[50]

frequently co-isolated with *P. aeruginosa* from the lungs of cystic fibrosis patients. Previous studies reported that the treatment of *S. aureus* with HQNO results in the development of resistance to aminoglycoside antibiotics. However, it was shown that HQNO inhibits respiration of *S. aureus* through the disruption of electron transport, a process required for the bacteria to be sensitive to aminoglycoside antibiotics. As a result, the surviving *S. aureus*, present in the form of small colony variants (SCVs), are inherently resistant to aminoglycoside antibiotics. Historically, it has been believed that *P. aeruginosa* simply outcompetes *S. aureus* in the CF lung, due to the fact that the number of *S. aureus* cultures isolated from CF lungs decreases as *P. aeruginosa* cultures increase. Yet, the ability of HQNO to essentially select for SCVs draws attention to the importance of *S. aureus* as an under-appreciated pathogen in settings where *P. aeruginosa* is present, particularly in the lungs of CF patients, where colonies of antibiotic resistant *S. aureus* may be growing undetected due to the presence of *P. aeruginosa*. Thus, in scenarios where clinical treatments are focused on *P. aeruginosa*, *S. aureus* will be able to persist and exert its effects on the host.

Recent evidence suggests *P. aeruginosa* also uses its AHL-based QS system, albeit in an indirect fashion, to mediate competition with other species. This strategy is based on the rearrangement of 3-oxo-C_{12}-homoserine lactone (3-oxo-C_{12}-HSL) to a tetramic acid derived product termed C_{12}-TA.[23] This rearrangement occurs through an irreversible, spontaneous Claisen-like reaction, which opens the lactone ring and forms the 3-acetyl-2,4-pyrrolidinedione heterocycle characteristic of tetramic acids (Fig. 7). Initial investigations of the rearrangement of 3-oxo-C_{12}-HSL to C_{12}-TA were performed *in vitro*, but the formation of significant quantities of C_{12}-TA has been hypothesized based on reports of AHL concentrations in biofilms reaching 600 μM and the rate of conversion of 3-oxo-C_{12}-HSL to C_{12}-TA.[24] This is relevant considering the time frame of *P. aeruginosa* infection in the lungs of CF patients, which can last for up to 30 years.

Tetramic acid derived natural products have been shown to possess a variety of biological activities, particularly as antimicrobial agents. C_{12}-TA was shown to have cytotoxic activity against a series of Gram-positive bacteria, but interestingly no activity was observed against Gram-negative bacteria, including *P. aeruginosa*. A notable example of clinical relevance is the susceptibility of *S. aureus* to C_{12}-TA. As described above, *P. aeruginosa* displaces *S. aureus* in the lungs of CF patients, a process in which the formation of C_{12}-TA may play a role. In light of current work with 4Q molecules (*vide supra*), the generation of C_{12}-TA may also play a role in the formation of SCVs of *S. aureus* as it exhibits similar bactericidal activity to the 4Q molecules in its effectiveness against Gram-positive bacteria. The presence of C_{12}-TA in *P. aeruginosa* biofilms, coupled with its antibacterial activity against potentially competitive bacteria, likely promotes the survival of *P. aeruginosa* in natural settings. Thus, a dual role for QS in *P. aeruginosa* can be imagined in which 3-oxo-C_{12}-HSL is used for intercellular communication, particularly in the formation of biofilms, whereas AHL-derived C_{12}-TA may be used to gain a competitive advantage over invading bacteria.

Another QS interspecies signaling approach used by *Pseudomonas aeruginosa* is dipeptide-derived diketopiperazines (DKPs), which can modulate quorum-dependent phenotypes in several different species of bacteria (Fig. 8). Interestingly, these DKPs were shown to activate LuxR based reporter assays, but found to inhibit swarming motility in *Serratia liquefaciens*, implying several functions for this class of compounds. Furthermore, as these DKPs were also found in *Proteus mirabilis*, *Citrobacter freundii*, and *Enterobacter*

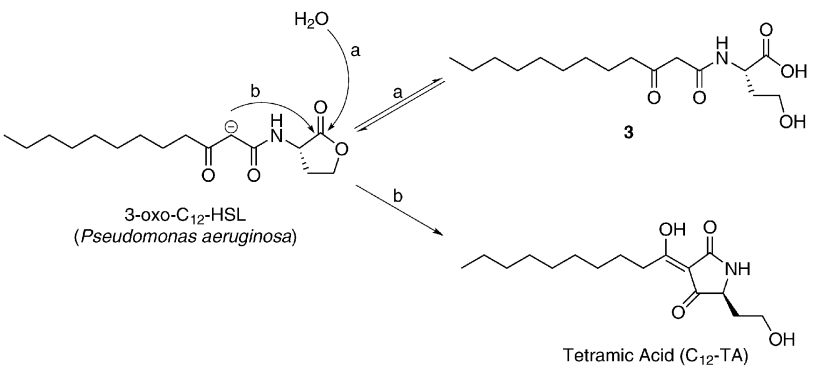

Fig. 7 Conversion of 3-oxo-C_{12}-HSL to C_{12}-TA *via* an irreversible Claisen-like reaction (pathway b). 3-oxo-C_{12}-HSL also undergoes reversible spontaneous hydrolysis to form **3** (pathway a).

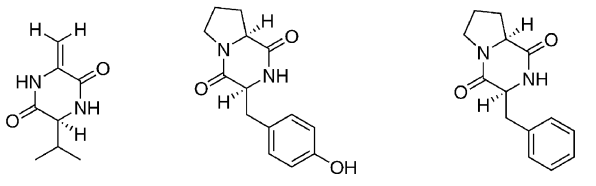

Fig. 8 Structures of diketopiperazines.

agglomerans, they may represent another potential avenue for communication across bacterial species.[25] However, as yet this hypothesis remains untested.

Prokaryote-to-eukaryote interactions

As stated, QS has evolved as a means for bacteria to coordinate behavior and function and thus organize a concerted effort for the interaction with multicellular organisms. In addition to helping bacteria organize their behaviors and functions, research is emerging that implicates QS as a means for bacteria to directly interact with eukaryotes through the action of their autoinducers. Similar to bacterial interspecies relations, the interkingdom signaling mediated by QS systems may occur either through recognition of bacterial signals by host cells or through the unregulated action of an autoinducer on the host cell.

QS and plants

The first example of QS based communication between prokaryotes and eukaryotes was discovered in a relationship between a marine bacteria and the green seaweed *Enteromorpha*.[26] It had been previously demonstrated that *Enteromorpha* zoospores would attach to marine bacterial biofilms (*Vibrio anguillarum*) and begin growth. However, if the conditions encountered by the seaweed were not ideal, the zoospores detached and continued a planktonic lifestyle. Based on the observation that the seaweed attached to single bacterial cells in the biofilm, it was hypothesized that *Enteromorpha* detect the presence of the bacteria through autoinducer recognition. *V. anguillarum* is a Gram-negative bacteria that uses three AHLs in the regulation of its QS system, 3-oxo-C_{10}-HSL, 3-hydroxy-C_6-HSL, and C_6-HSL. To show the necessity of each autoinducer, several QS mutants lacking the synthases for each AHL were created. When biofilms of each knockout strain were grown, *Enteromorpha* zoospores failed to adhere, suggesting a crucial role for the recognition of the AHL signals by the seaweed. However, this did not rule out the possibility that the bacteria may have developed an unpredicted phenotype during creation of the QS mutants. To account for this, the authors used an elegant approach in which *E. coli* were transformed with the genes required for synthesis of the three AHLs to show that the seaweed responds specifically to the AHLs of *V. anguillarum*. *Enteromorpha* attachment was observed only on those *E. coli* biofilms expressing the genes for AHL synthesis, implicating a direct response of the seaweed to the signals. Finally, in biofilms lacking the AHL synthases, synthetic 3-oxo-C_{10}-HSL, 3-hydroxy-C_6-HSL, and C_6-HSL could be added to the medium and attachment was restored, pointing towards a direct role of the AHL signals in the attachment of zoospores to the biofilms.

Another example of eukaryotic gene expression in response to bacterial QS signals was demonstrated in the legume *Medicago truncatula*.[27] *M. truncatula* forms relationships with both symbiotic and pathogenic bacteria, including two known AHL-producing bacteria, *Sinorhizobium meliloti* and *P. aeruginosa*. Proteomic analysis was performed on root cells exposed to AHLs produced by each bacteria, 3-oxo-$C_{16:1}$-HSL in *S. meliloti* and 3-oxo-C_{12}-HSL produced by *P. aeruginosa*, and 154 proteins were found to be upregulated. Interestingly, about two-thirds of the proteins showed similar upregulation patterns in response to both autoinducers. However, the remaining one-third showed distinct patterns in response to each signal, a finding that led to the conclusion that the plant may, in fact, have a mechanism to distinguish between the two signals. Although the exact response of the plants to the autoinducers was not determined, it is important to note that the legume did not exhibit browning or necrosis associated with toxicity, even when exposed to high concentrations of AHL. This is in opposition to the effects of AHLs often exerted on mammalian cells, in which apoptosis or inflammation is observed (*vide supra*).

AHL-based immunomodulation

The first example of QS signaling molecules directly exerting their effects on host cells was reported by Telford *et al.*[28] In this study, 3-oxo-C_{12}-HSL was found to inhibit production of the cytokines interleukin-12 and tumor necrosis factor alpha (TNF-α) by LPS-stimulated macrophages. Since this first report, several studies have detailed the effects of AHL-based signaling molecules, particularly the 3-oxo-C_{12}-HSL signal secreted by *P. aeruginosa*, on human cells. Responses of host cells range from modulation of the inflammatory response, depression of the host defense response, and induction of apoptosis. These studies have been the subject of several reviews.[29,30]

Several studies have provided evidence that point to the recognition of AHLs as ligands of mammalian receptors. Recent work by Kravchenko *et al.* shows that 3-oxo-C_{12}-HSL acts as a negative regulator of host innate immune responses.[31] In this study, several biochemical markers of 3-oxo-C_{12}-HSL effects in mammalian macrophages, along with morphological changes in cell organelles, were observed. Interestingly, these effects occurred in a wide variety of mammalian cell types and absolutely required the structural integrity of the lactone ring motif and its natural stereochemistry, as these changes in mammalian cells were not induced by either hydrolyzed 3-oxo-C_{12}-HSL or 3-oxo-C_{12}-HSL with inverted stereochemistry thereby suggesting the existence of a specific receptor for 3-oxo-C_{12}-HSL in mammalian cells. Evidence for the type of receptor was found in a study in which 3-oxo-C_{12}-HSL induces apoptosis of fibroblasts *via* calcium mobilization from the ER to the cytoplasm. However, when fibroblasts were treated with inhibitors of phospholipase C, an enzyme crucial to signaling *via* G-protein coupled receptors (GPCRs), apoptosis was not induced. As such, this study implicates the potential for AHL recognition by

GPCRs.[32] Furthermore, GPCRs are good candidates for AHL receptors based on their ability to bind a variety of hormones and medicinal drugs in addition to their role in intracellular signaling through interactions with phospholipase C.[33]

B. Subtilis and CSF

In contrast to the detrimental effects caused by the QS signals of *P. aeruginosa*, a study by Fujiya *et al.* suggests a cooperative bacteria-host relationship mediated by bacterial QS.[34] *Bacillus subtilis* is a Gram-positive bacteria that is part of the human enteric flora, and several reports have suggested possible health benefits resulting from the presence of *B. subtilis* in the gastrointestinal tract. *B. subtilis* employs a pentapeptide to regulate the expression of competence and sporulation, termed CSF (competence and sporulation factor), that functions intracellularly after active import by *B. subtilis*. The proposed probiotic role of *B. subtilis* was reinforced by a study in which CSF activates two crucial kinase-dependent survival pathways in intestinal epithelial cells. Also in response to CSF, intestinal epithelial cells avert cell injury and loss of barrier function through the expression of cytoprotective heat shock proteins. Interestingly, these effects are moderated by CSF through its uptake by epithelial cells *via* an apical membrane organic cation transporter-2 (OCTN2).[34] This finding led the researchers to propose a role for OCTN2 in the survey and detection of changes in the enteric microbiome, which may help the host to regulate intestinal homeostasis.

Eukaryotic response

QS and fungi

QS-mediated interkingdom interactions also occur between microorganisms in health care settings, as in the case of *P. aeruginosa* and the diploid fungus *Candida albicans*. Although infections involving methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp (VRE) receive the most attention regarding hospital-acquired diseases, infections by both *C. albicans* and *P. aeruginosa* often originate from medical devices in hospital infection control units. As discussed previously, *P. aeruginosa* presents major problems in CF patients, but studies have also shown that *C. albicans* can be routinely isolated from the sputum of CF patients, even though resulting complications are rare. However, the control of *P. aeruginosa* by administering antibiotic therapies to CF patients often results in increased incidence of *C. albicans* infections, suggesting a competitive relationship between *P. aeruginosa* and *C. albicans* in the lungs of CF patients.[35] In this vein, Hogan and Kolter have shown the hyphal form, but not the yeast form, of *C. albicans* exhibits attenuated proliferation in the presence of *P. aeruginosa*.[36] 3-oxo-C_{12}-HSL is at least partly responsible for this phenomenon, as it inhibits filamentation in *C. albicans* at concentrations above 50 μM.[37] Conversely, farnesol, a compound produced by *C. albicans*, significantly impacts synthesis of PQS by *P. aeruginosa* when present at concentrations greater than 25 μM.[38] The inhibition of PQS synthesis also results in the attenuated production of pyocyanin, a compound which is toxic to *C. albicans*. Interestingly, accumulation of farnesol in dense cultures of *C. albicans* inhibits hyphal growth, and consequently has been referred to as the first QS system to be discovered in eukaryotes.[39] This example of microbial warfare does not represent findings simply in laboratory settings, but is supported by clinical observations. Especially pertinent to the discussion herein is the response to bacterial QS by a eukaryote, and the resulting interplay of the two QS systems as an example of the use of autoinducers as weapons in microbial conflict.

Plant-produced brominated furanones

Perhaps the most studied eukaryotic response to QS is represented by the secretion of brominated furanones by the red algae *Delisea pulchra*. Marine dwelling plants are often colonized by bacteria in the form of biofilms, which often leads, in turn, to macrofouling by higher organisms. However, *D. pulchra* was found in marine environments to be relatively free from fouling organisms, and microscopy revealed bacterial populations to be reduced on the surface of *D. pulchra* relative to neighboring plants. Givskov *et al.* found that the alga was releasing non-toxic metabolites at its surface that are responsible for the lack of fouling. The compounds were found to be a series of brominated furanones, which inhibited multiple AHL-dependent processes including swarming motility in *Serratia liquefaciens* and bioluminescence in *V. harveyi* and *V. fischeri* (Fig. 9).[40] Importantly, no growth inhibition was observed during these experiments, indicative of selective inhibition of QS rather than simply bactericidal activity.

Since their discovery, the activity of brominated furanones has been explored extensively, and naturally occurring furanones have been found to inhibit both AHL and AI-2 based QS in *Vibrio* sp. Inhibition of these two QS system was rationalized based on the structural similarity of the halogenated furanones to the lactone and tetrahydrofuran rings characteristic of the AHLs and AI-2s, respectively. Because of this similarity, the furanones were initially believed to compete with the natural autoinducers for binding to their cognate receptors.[41] However, this notion was discounted based on several reports concerning QS inhibition in *V. fischeri*, where halogenated furanones were demonstrated

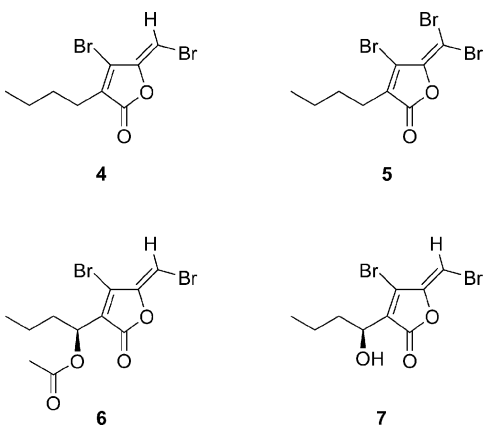

Fig. 9 Structures of naturally occurring brominated furanones produced by *D. pulchra*.

to destabilize and accelerate turnover of the transcriptional activator $LuxR_{Vf}$.[42] Similar activity was seen in the inhibition of QS in *V. harveyi*, as the furanones rendered the QS master regulator protein $LuxR_{Vh}$ unable to bind DNA and initiate gene transcription.[41] Interestingly, $LuxR_{Vf}$ and $LuxR_{Vh}$ are not homologous, but, in light of the structures of the furanones and their chemical reactivity it is likely that a similar mode of inhibition is active for both. Because of the presence of electrophilic centers in the furanones, it is possible that the furanones become chemically linked to both $LuxR_{Vf}$ and $LuxR_{Vh}$ through various mechanisms (*e.g.*, Michael addition at the 1,4-unsaturated ketone, nucleophilic addition to the ketone, or halogen substitution). A common mode of action would be encouraging for the development of antimicrobial strategies against human pathogens, as homologs of $LuxR_{Vh}$ have been found in other *Vibrio* species, including the human pathogens *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. Thus, the prevalence of a common target implicates a potential for halogenated furanones as a broad spectrum inhibitor for the treatment of disease.[41]

Due to their QS inhibitory effects, halogenated furanones have shown practical potential for the treatment of disease in gnotobiotic shrimp, which are prone to infection by *V. harveyi*, *V. campbellii* and *V. parahaemolyticus*.[43] Treatment of bacterial infection in aquaculture has seen heavy dependence on the use of antibiotics, and, consequently, the rise of antibiotic resistance. Importantly, it is for this same reason that QS represents an attractive target in the treatment of human disease. Indeed, Defoirdt *et al.* have shown that treatment of the shrimp *Artemia nauplii* with synthetic furanone **4** protected the shrimp from death induced by exposure to *V. harveyi* and *V. campbelli*, at similar concentrations needed for QS disruption in *V. harveyi*.[43] The discovery of these QS inhibitors that are structurally similar to AHLs has also inspired investigations into their use against human pathogens, particularly *P. aeruginosa*.[44] In fact, halogenated furanones were shown to attenuate the virulence and accelerate biofilm clearance of *P. aeruginosa* in mouse models, lending further credence to their proposed function as a treatment for bacterial infections.[45]

QS modulation has also been discovered in higher plants (*i.e.*, plants with a vascular system), which have been found to secrete AHL mimics. As described, the legume *M. truncatula* alters its gene expression in response to AHLs (*vide supra*). Gao *et al.* have shown that *M. truncatula* also responds to bacterial communication through the production of its own small molecule AHL-mimics.[46] Although the structural identities or mechanistic details of these entities remain shrouded, reporter assays have been used to show that these compounds both inhibit and stimulate QS expression in AHL-sensitive bacteria. This finding leads to the idea of a more interactive relationship than those seen between the seaweed *Delisea pulchra* and AHL-producing bacteria, in which the eukaryotic response represented an attempt to disrupt the bacterial communication.

Human response to bacterial QS

Regulation of QS genes may also be controlled by human signals, as in the case of *P. aeruginosa*, which binds the cytokine interferon-γ (IFN-γ). Binding occurs through OprF, an outer membrane protein, and activates the QS machinery and expression of the virulence factor PA-I lectin.[47] This example contrasts to the previous discussion involving *M. truncatula*, which secretes AHL mimics, in that the primary role of IFN-γ is not for mediating communication with bacteria. However, the main function of IFN-γ is for bacterial clearance, which suggests this bacterial system evolved in response to host immune activation. Furthermore, OprF is conserved among many bacteria, suggesting a role in the recognition of host signals by many bacteria.

Bacteria have also been found to respond to small molecule signals utilized by humans in the form of epinephrine and norepinephrine (Fig. 10(A)).[48] Sperandio *et al.* have described the *qseA* and *qseBC* regulatory systems in enterohemorrhagic *E. coli*. QseA regulates the locus of enterocyte effacement (LEE) pathogenicity island, and QseBC regulates the activation of flagella and motility genes. Initially, this system was proposed to be under the control of AI-2-based QS, as LuxS mutants were unable to initiate transcription of the LEE genes. However, when these QS mutants were incubated with human cells, expression of these genes was restored, leading to the hypothesis of a eukaryotic signal interacting with the bacterial AI-2 QS system.[48]

Guided by the knowledge of hormone-based mammalian intercellular signaling, the presence of epinephrine (Epi) and norepinephrine (NE) in high physiological concentrations (50 μM) and the fact that norepinephrine (NE) is taken up by bacteria, the authors reasoned that Epi and NE may be involved in this activation. Indeed, treatment of *E. coli luxS* mutants with purified Epi and NE resulted in the restoration of LEE activity. However, based on structural differences between Epi/NE and AI-2, it seemed unlikely that Epi/NE was recognized through the AI-2 pathways. Indeed, the supplementation of *E. coli luxS* mutants with purified AI-2 failed to restore expression of the LEE genes. Thus, further study led to the discovery of an additional class of autoinducers produced by *E. coli*, called AI-3, although its structure remains unsolved.

Based on the interactions between the AI-3 system of *E. coli* with the human Epi/NE system, AI-3 has been proposed to be an interkingdom signal. In support of this notion, Clarke *et al.* have purified the *E. coli* QseC receptor and shown that it responds to activation by both AI-3 and Epi/NE (Fig. 10(B)).[49] An *in silico* screen of the sequence of the QseC also revealed sequence homology between the receptor in *E. coli* and proteins in several other bacteria, including other enteropathogens such as *Shigella* sp. and *Salmonella* sp. Based on this sequence homology, a potential role of AI-3 in interspecies signaling among bacteria, as well as interkingdom between bacteria and humans, can be envisioned.

Conclusions and outlook

In nature, bacteria are almost always found in diverse communities comprised of many different species, including both prokaryotes and eukaryotes. Thus, there is a clear advantage to be gained through concerted actions regulated by QS within a bacterial species. However, it is becoming apparent that QS

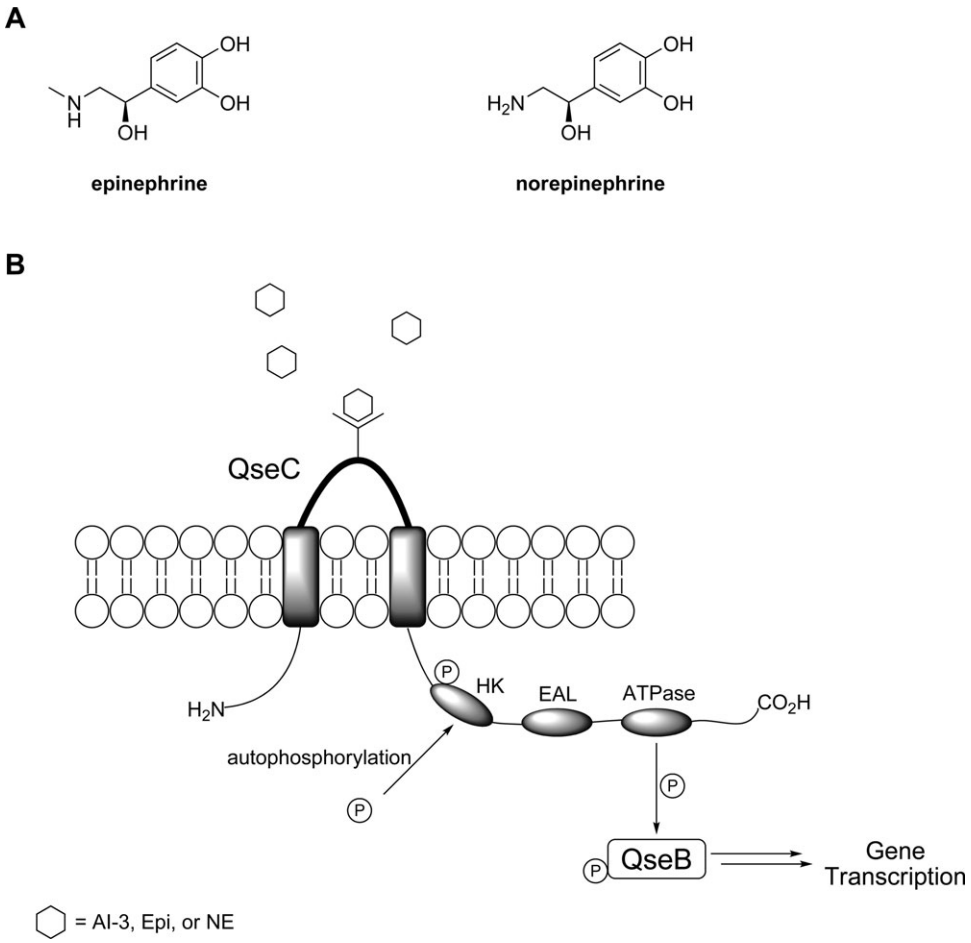

Fig. 10 (A) Structures of epinephrine and norepinephrine. (B) Model of QseC and its role in sensing Epi/NE and AI-3. QseC contains a histidine kinase (HK) region which autophosphorylates upon binding of AI-3, Epi, or NE. The ATPase region is responsible for transferring phosphate to QseB, which serves as the transcriptional activator of *qseB* and the flagella regulon *flhDC*. QseC also contains an EAL domain, which is proposed to confer cyclic diguanylate phosphodiesterase activity.

systems also play roles in both communication and competition *between* bacterial species. Interspecies communication systems have been proposed to exist in a wide variety of bacteria, but the capacity of these systems to mediate interspecies relations has only been demonstrated in a handful of bacteria. In a similar vein, QS-based systems also impart a competitive advantage through antibacterial action of the autoinducers, but the importance of these phenomena *in vivo* remains to be seen. As such, future work will undoubtedly focus on the roles of both communication and competition in biological settings, particularly in the context of clinical scenarios. Perhaps of greater consequence, at least in assessing and combating bacterial pathogenesis, is the role of QS interactions with eukaryotic hosts. As described, bacterial QS has the potential to exert its effects on mammalian cells, and understanding this process may help in the development of antimicrobial strategies. A likely lead towards this end may be found in the eukaryotic hosts themselves, as certain eukaryotes have the potential to respond to, and disrupt, bacterial communication. In total, because of the many relationships that can be mediated, QS may represent a more global language of communication that spans across every kingdom of life and human interpretation of this language will impart a deeper knowledge of prokaryotic lifestyles and provide the opportunity for an appropriate response.

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