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REVIEW

Bacterial quorum sensing and interference by naturally occurring biomimics

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Abstract Bacteria are able to coordinate gene expression as a community through the secretion and detection of signalling molecules so that the members of the community can simultaneously express specific behaviours. This mechanism of regulation of behaviour appears to be a key trait for adaptation to specific environments and has been shown to regulate a variety of important phenotypes, from virulence factor production to biofilm formation to symbiosis related behaviours such as bioluminescence. The ability to communicate and communally regulate gene expression is hypothesised to have evolved as a way for organisms to delay expression of phenotypes until numerical supremacy is reached. For example, in the case of infection, if an invading microorganism were to express virulence factors too early, the host may be able to mount a successful defence and repel the invaders. There is growing evidence that bacterial quorum sensing (QS) systems are involved in cross-kingdom signalling with eukaryotic organisms and that eukaryotes are capable of actively responding to bacteria in their environment by detecting and acting upon the presence of these signalling molecules. Likewise, eukaryotes produce compounds that can interfere with QS systems in bacteria by acting as agonists or antagonists. An exciting new field of study, biomimetics, takes inspiration from nature's models and attempts to design solutions to human problems, and biomimics of QS systems may be one such solution. This article presents the acylated homoserine lactone and autoinducer 2 QS systems in bacteria, the means of intercepting or interfering with bacterial QS systems evolved by eukaryotes, and the rational design of synthetic antagonists.

Keywords Quorum sensing · *N*-acylated homoserine lactone · Autoinducer 2 · Virulence · Quorum sensing antagonist · Biomimic

"The proof of evolution lies in those adaptations that arise from improbable foundations"—Stephen Jay Gould

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Introduction

Organisms must accomplish life's requirements within the confines and constraints of their environment, and as a consequence either adapt or go extinct. Adaptations ultimately lead to genetic changes that sustain the species in the long term. Some bacteria have evolved elaborate systems whereby they are able to communicate via the production and reception of chemical cues, a phenomenon termed *quorum sensing*. Quorum sensing (QS) involves the regulation of gene expression in response to extracellular autoinducer (AI) molecules. Bacterial cells produce and release AIs, and when the concentration reaches a threshold, the bacterial population as a whole alters gene expression, which may be involved in pathogenesis, community assembly (biofilm formation) or the modulation of association with higher organisms. It is suggested that QS regulatory systems ensure that pathogenic traits are only expressed when the bacterial population density is high enough to overwhelm the host. For example, in the plant pathogen *Pantoea stewartii*, mutations in the LuxR homologue EsaR cause early and increased expression of

virulence genes, as EsaR normally acts as a repressor, but these mutants are avirulent when inoculated into the plant, in spite of the increased virulence factor expression [1]. It has been suggested that the early expression of virulence factors allows the plant to recognise the presence of the pathogen and mount a successful defensive response. Thus the timing of gene expression through QS is important, although in a social, not a temporal, sense.

Bacterial quorum sensing systems

A number of different QS systems exist. In general, Gram-positive bacteria use peptide signals which are perceived through membrane-bound receptor-histidine kinases, while Gram-negative bacteria use small molecules which diffuse across the cytoplasmic membrane and bind to regulatory proteins within the cell. This review will focus on two QS systems, the acylated homoserine lactone and autoinducer 2 QS systems, although many more examples of QS systems exist, including systems using oligopeptides, quinolones, cyclic dipeptides, and γ-butyrolactones as signals.

Acylated homoserine lactone QS

Approximately 7% of Gram-negative bacteria within the alpha, beta and gamma proteobacteria groups utilise acylated homoserine lactones (AHLs) as AIs. This system was first described in *Vibrio fischeri*, where it regulates bioluminescence [2, 3]. In *V. fischeri*, two proteins—Lux I, the AI synthase, and LuxR, the transcriptional regulator that is activated by the AI—regulate the luciferase operon. The AHL synthases produce AHL molecules that have a conserved homoserine lactone ring (derived from *S*-adenosyl methionine) with a variable acyl chain (derived from lipid metabolism) attached by an amide bond (Fig. 1a). AHLs have been described with acyl chains of 4–18 carbons, and they can vary in oxidation state and degree of unsaturation [4]. The LuxR proteins act as transcription factors and have two domains: the N-terminal region required for AHL binding, and the C-terminal DNA-binding region. Once AI is bound to the LuxR receptor, the AI/LuxR complex binds DNA and activates expression of QS-controlled genes. The AI-receptor interaction shows some specificity such that each receptor will only bind to its cognate AI and a limited variety of closely related AIs.

Pseudomonas aeruginosa has multiple hierarchical QS systems that regulate several virulence factors. *P. aeruginosa* produces two AHL molecules: *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) (Fig. 1a) and *N*-butanoyl-L-homoserine lactone (C4-HSL) that bind to LasR and RhlR respectively and do not cross-react. The LasR/AI complex activates transcription of *rhlR* and *rhlI*. The expression of many virulence genes in *P. aeruginosa* requires LasR and/or RhlR, activated by their respective AI molecules [5], and mutations in *lasI* and *rhlI* signal

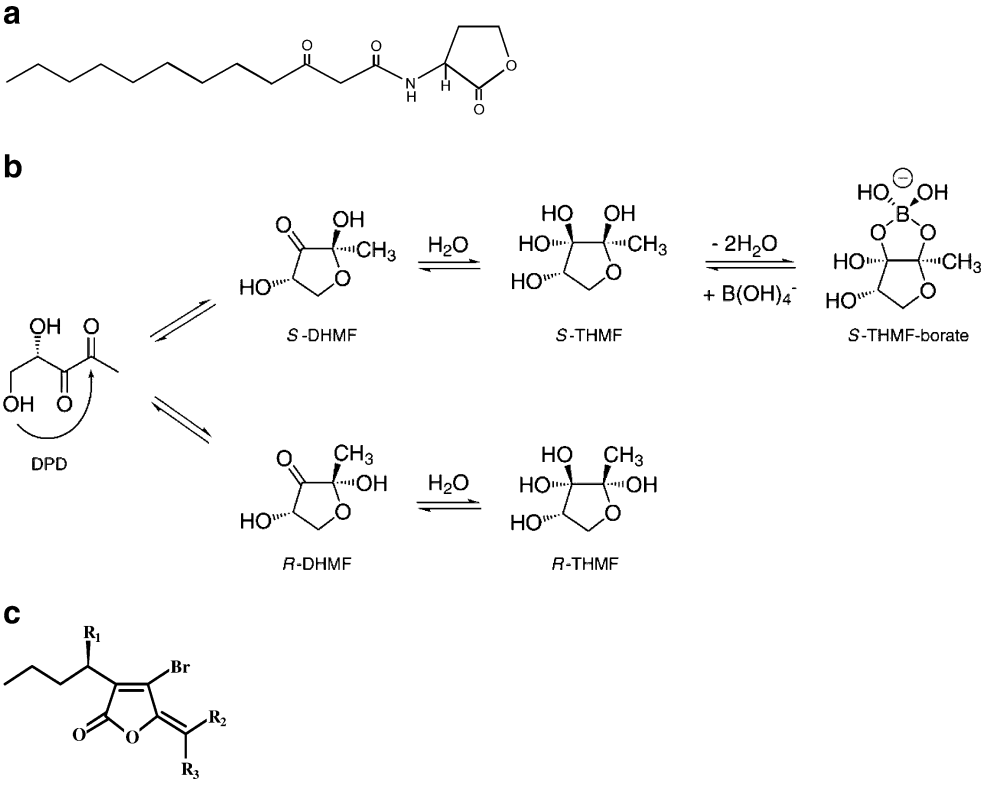

Fig. 1 Structure of QS molecules and a biomimic. **a** *N*-(3-oxododecanoyl)-L-homoserine lactone; **b** the molecules collectively called AI-2. The precursor molecule, DPD, can form two active AI-2 signal molecules. The *S*-THMF-borate is used by *V. harveyi* as a signal, and the *R*-THMF is used by *Salmonella enterica* serovar Typhimurium (from [81]; reprinted with the permission of the AAAS). **c** Representative structure of furanones produced by *D. pulchra*, where R groups can be different substituents

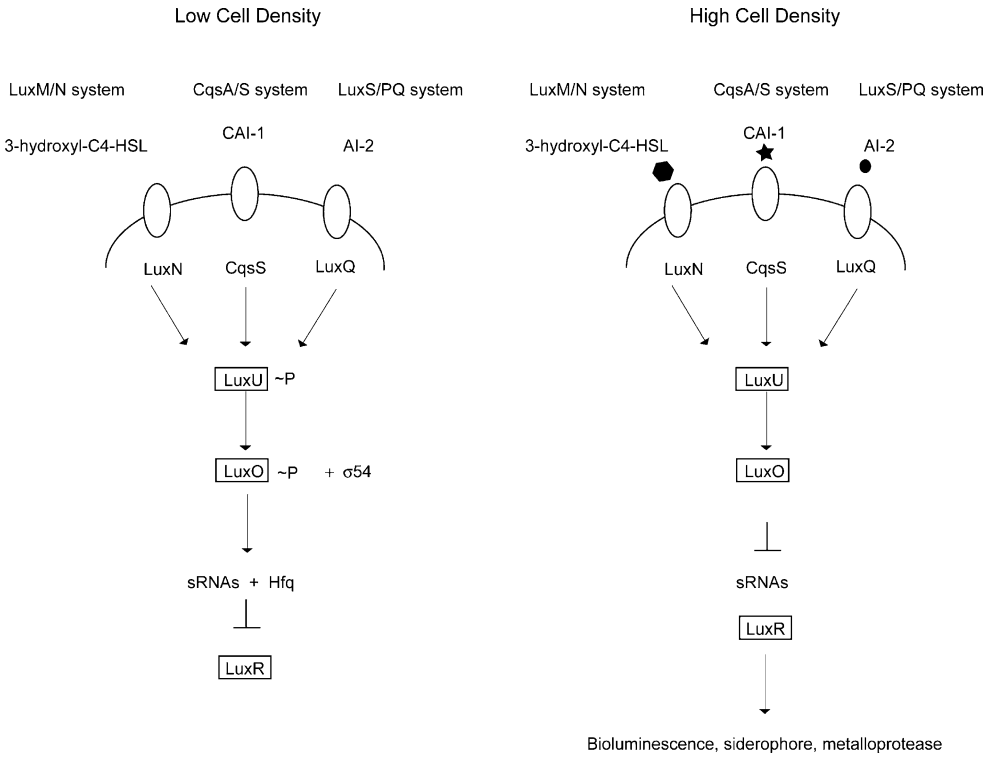

Fig. 2 Model of the QS system in *V. harveyi*. At low cell density, in the absence of autoinducers, the sensor kinases LuxN, LuxQ and CqsS autophosphorylate. The phosphorelay cascade results in the phosphorylation of LuxU and LuxO. Phosphorylated LuxO is activated, and with sigma 54, it activates the expression of sRNAs. The sRNAs, along with Hfq, destabilise the *luxR* mRNA. Under conditions of high cell density, interaction of the autoinducers stimulates the LuxN, LuxQ and CqsS sensors to switch from kinase to phosphatases. This results in the dephosphorylation of LuxO and the inactivation of its function. Inactivation of LuxO allows the activation of the *lux* operon by LuxR

synthase genes strongly reduce virulence in several animal models (reviewed in [6]). Further, a third LuxR homologue has been identified, QscR, which acts to repress transcription of *lasI* [7]. LasR, RhlR and QscR each control their own regulons, some of which overlap [8]. In addition, QscR can form homomultimers in addition to forming heteromultimers with LasR and RhlR [9], and can bind to several AHLs including 3-oxo-C12-HSL [10], indicating that there can be cross-talk between closely related AIs. Thus, in *P. aeruginosa*, there is a regulatory hierarchy whereby LasR regulates *rhlR* and LasI regulates QscR.

In *V. fischeri* and *P. aeruginosa*, the receptor pocket of the mature LuxR protein is accessible and reversibly binds to the AI molecule. This binding is thought to cause a conformational change in the receptor protein, which affects its ability to form dimers or multimers and to subsequently bind to promoters. In the majority of cases, binding of the AI to the receptor leads to induction of target genes; however, in *Pantoea stewartii* the LuxR homologue EsaR binds to target sequences in the absence of AI, which acts as repressor. DNA binding is reversed by AI interaction with EsaR which then targets the protein for proteolysis [11]. In contrast, the LuxR homologue in *Agrobacterium tumefaciens*, TraR, only folds properly when it is synthesised in the presence of its cognate AI [12], and TraR lacking the AI molecule is rapidly degraded; the receptor pocket is not accessible in the folded receptor and hence the signal does not dissociate, in contrast to *V. fischeri* and *P. aeruginosa*, where the signal does dissociate. The TraR/AI complex exists in the cell primarily as dimers and binds DNA as a dimer [13]. These differences indicate that *V. fischeri* will stop the induction of QS-regulated genes immediately upon signal limitation, while in *A. tumefaciens*, QS-regulated gene expression will continue until all the available TraR/AI complex has been degraded. A further caveat to these systems is the fact that RhlR binds to *rhlAB* in both the presence and absence of its AI, and acts as a repressor in the absence of AI, but as an activator when bound to AI [14].

In some cases, bacteria are unable to synthesise their own signal, but can respond to signals of other bacteria—a form of eavesdropping. *Escherichia coli* has the ability to alter gene expression and phenotype in response to AHLs produced by other bacterial species by means of SdiA, a LuxR homologue for which there is no cognate AI synthase [15]. Genes affected by the presence of AHLs are involved in amino acid transport and metabolism as well as transcriptional regulation. Similarly, *Burkholderia cepacia* is able to perceive and respond to AHLs produced by *P. aeruginosa*, which may have implications for cystic fibrosis patients with co-infection of the two organisms [16].

Autoinducer-2 QS

A QS system found in a wide range of bacterial species is the autoinducer-2 system (AI-2), first described in *Vibrio harveyi* [17, 18]. Due to its occurrence in Gram-positive

and Gram-negative bacteria, it has been suggested that this signalling system was present before the divergence of those organisms [19]. The AI-2 synthase, called LuxS, produces the AI-2 precursor, 4,5-dihydroxy-2,3-pentadione (DPD). DPD can spontaneously cyclize to generate a number of forms (see [20]), which are collectively referred to as AI-2 (Fig. 1b). DPD is able to form 2,4-dihydroxy-2-methylhydro-3-furanone (DHMF) in two forms (*R* and *S*). *S*-DHMF can form *S*-THMF, which forms a diester containing a boric acid, the first identified AI-2 signal, which binds to the signal receptor LuxP in *V. harveyi* [21]. The *R*-DHMF hydrates to form *R*-THMF, which is the form that binds to the LsrB receptor protein in *Salmonella enterica* serovar Typhimurium [22, 23].

The AI-2 system is best understood in *Vibrio* spp., which serve as model organisms for AI-2 signalling (Fig. 2). In *V. harveyi*, in addition to the AI-2 system, there is an AHL system that has no similarity to the LuxI/R system, and a third system, CqsA/S, which is only found in this genus. These systems are coordinately regulated and signal transduction occurs through a two-component system with all three pathways converging at a common point. Briefly, at low cell density (and therefore low AI concentration), the sensor proteins autophosphorylate and transfer phosphate to the shared phosphotransferase protein, LuxU, which in turn phosphorylates the response regulator, LuxO. Phosphorylated LuxO is active and, in conjunction with sigma 54, activates the expression of genes encoding small regulatory RNAs. The sRNAs, along with the sRNA chaperone Hfq, bind to the mRNA encoding the activator protein LuxR and destabilise it; hence the QS system is in the "off" position. At high cell density, the signals bind to their cognate sensors, resulting in the switching of the receptor proteins from kinase to phosphatase activity, which leads to the inactivation of LuxO and therefore the activation of LuxR, in order to control target gene expression.

While the AI-2 system occurs in a wide range of both Gram-negative and Gram-positive organisms, the receptor for the AI-2 signal, LuxP, has only been identified in *Vibrio* spp., indicating that other species may not have evolved to utilise this compound as a true signal. In fact, the only genes known to be regulated by the AI-2 system in other species encode an ABC transporter, Lsr, which is responsible for the uptake of the AI-2 signal in *S. typhimurium* and *E. coli* [23–25]. After uptake, the AI-2 signal is phosphorylated and interacts with LsrR, which then represses the *lsr* operon.

Synthesis of AI-2 is a by-product of the activated methyl cycle (AMC), which is responsible for the generation of the major methyl donor *S*-adenosylmethionine (SAM) and the recycling of methionine. The AMC recycles the main methyl donor, SAM by converting it to *S*-adenosyl-L-homocysteine (SAH), which is detoxified by the Pfs enzyme, forming adenosine and *S*-ribosyl-homocysteine (SRH). It has been argued that the effects of LuxS mutations seen in a number of bacterial species are the result of interruptions to the AMC [26], so altered gene expression in mutants may be due to the interruption of the metabolic pathway and not due to QS as such. LuxS mutants will accumulate SRH and the intracellular levels of homocysteine will drop. Methionine synthesis will also be affected, as will other amino acid synthesis pathways, since homocysteine is recycled to form methionine. It is therefore important to use purified AI-2 signal in studies to demonstrate the specific effects of the signal and to discriminate these from potential secondary effects. For example, addition of purified AI-2 to a co-culture of a *Staphylococcus aureus luxS* mutant and the isogenic wild type strain did not complement the growth defect of the mutant strain, unlike the case when the mutation was complemented by *luxS*-containing plasmid [27]. This indicates that the growth defect was not due to the diffusible AI molecule. Thus, it is clear that the AI-2 system in *Vibrio* spp. has the hallmarks of a true QS system, including the possession of an AI synthase, signal receptors and signal transduction cascade. Further work will be required to determine whether LuxS plays a role in the production of AIs in other species, or whether its role is a metabolic one.

Communication in a complex world

Clearly there are systems that bacteria use to communicate and coordinate gene expression in order to provide a competitive advantage in nature. However, bacteria in the environment must compete with other bacteria and with higher organisms, some of which have similar communication systems and others that have coevolved to exploit or circumvent the bacterial QS response. These interactions can be symbiotic, agonistic, antagonistic or apparently accidental. Additionally, the QS systems are attractive targets for controlling bacterial behaviour, and thus strategies aimed at interfering with these would be useful for controlling virulence and colonisation. The following sections will explore QS as it relates to the interaction of the signalling population with other organisms and the development of QS interference strategies that exist in nature.

Interspecies signalling and interference

Given that bacteria have evolved the ability to communicate via QS, it is reasonable and even expected that they have also evolved the ability to compete with each other via QS. There

are examples of interference in which microbes release enzymes that degrade AI molecules. Two classes of AHL-degrading enzymes have been identified, the lactonases and acylases. *Bacillus* sp. release an AHL-lactonase, AiiA, that cleaves the lactone ring of AHL molecules and probably interferes with the AHL signalling of species competing with *Bacillus* in nature [28, 29]. *Bacillus* itself does not produce AHLs but instead uses peptide signals, and is therefore only degrading the signal of Gram-negative competitors. Because AiiA targets the AHL ring, it is nonspecific with regard to the acyl side chain and thus inactivates many AHLs. Heterologous expression of this gene in *P. aeruginosa* reduces endogenous AI concentration, virulence gene expression and swarming [30]. Furthermore, the expression of AiiA in *E. coli* attenuated the virulence of *Erwinia carotovora* in potatoes when the two organisms were co-cultured [29]. The *Variovorax paradoxus* lactonase is able to degrade and utilise *N*-(3-oxohexanoyl)-L-homoserine lactone as a sole source of energy and nitrogen [31]. Furthermore, Uroz et al. [32] isolated several bacterial species that produced lactonases from the tobacco rhizosphere, including *Pseudomonas* sp., *Variovorax* sp., *Comamonas* sp. and *Rhodococcus* sp. The degradation properties of various strains differed with respect to substrate preferences and degradation kinetics. For example, *Rhodococcus erythropolis* was able to inactivate a wide range of AHL molecules and was able to interfere with violacein production in *Chromobacterium violaceum*, the transfer of Ti plasmids, which are involved in pathogenicity in *A. tumefaciens*, and the pathogenicity of *E. carotovorum*. Homologues of AiiA have also been identified in *P. aeruginosa*, *Arthrobacter* sp., *A. tumefaciens* and *Klebsiella pneumoniae* [33–35]. The differences in specificity and activity of various lactonases may reflect the types of bacteria that are normally encountered in the environment, and strongly suggests co-evolution of the AHL quorum sensing and AHL degrading activities in those environments.

In addition to the AHL-lactonases, AHL-acylases that cleave the acyl side chain from the HSL ring have been identified. AiiD was identified in *Ralstonia* isolates, and *P. aeruginosa* expressing the AiiD protein showed reduced accumulation of 3-oxo-C12-HSL and C4-HSL, resulting in decreased expression of virulence factors and attenuated virulence in a *Caenorhabditis elegans* model [36]. *Streptomyces* sp. also produce an AHL-acylase that is more effective at degrading AHLs with acyl chains of six or more carbons, regardless of the substitutions [37]. *P. aeruginosa* produces an AHL-acylase which is capable of degrading the 3-oxo-C12-HSL but not the C4-HSL, indicating that these enzymes may also be important for the regulation of intracellular AI accumulation under certain environmental conditions [38]. These examples may represent strategies that enable bacteria to control QS behaviour.

Cross-kingdom signalling and interference

Bacteria co-exist with other organisms (both prokaryotic and eukaryotic), and the widespread occurrence of QS systems regulating a multitude of phenotypes in bacterial species has resulted in the evolution of higher organisms that are able to exploit or interfere with these signalling pathways. It is now recognised that cross-kingdom perception of AI molecules occurs and that other organisms modify their behaviour in response to these molecules. These interactions include true cross-kingdom symbiotic relationships whereby both organisms actively participate in signal production and reception, perception of cues where coincidental production of the signal is perceived by another, and chemical manipulation, where the production of the signal directs the behaviour of the receiver, benefiting the producer.

Symbiotic signalling

An example of a true cross-kingdom communication system would be the symbiotic relationship between the Hawaiian squid, *Euprymna scolopes*, and *V. fischeri*, which it harbours in its light organ. The squid light organ emits light produced by *V. fischeri* downward during nocturnal feeding, thereby hiding its shadow in an attempt to prevent detection by predators. This symbiotic relationship is dependent on the QS-regulated phenotype of bioluminescence in *V. fischeri* [39]. The development of normal crypt epithelium of squid light organ depends on colonisation by *V. fischeri*, and successful colonisation of the crypt by *V. fischeri* is dependent on a functional QS system as well as the QS-regulated genes [40]. In addition, the squid produces chitin oligosaccharides that serve as a chemotactic signal that directs *V. fischeri* migration into the light organ [41]. When native *V. fischeri* strains from the squid light organ compete with *V. fischeri* isolates from light organs of fishes, the native strain dominates, indicating co-evolution of this relationship between the squid and its native *V. fischeri* strain [42].

QS signals as cues

Cues are defined as the production of a signal that is perceived by another, benefiting the receiver but not the producer [43]. An example of a cue would be the colonisation of bacterial biofilms by higher organisms that can detect the production of AHLs by biofilm cells. Zoospores of the marine alga *Ulva intestinalis* show enhanced colonisation of surfaces in the presence of AHL-producing *Vibrio anguillarum* [44]. The *Ulva* zoospores respond to a range of AHL molecules and showed a preference for settling on surfaces releasing AHLs with acyl chains of six or more carbons, which may be a reflection of

their decreased diffusion rate. Motile zoospores of the green seaweed *Enteromorpha* also detect and respond to acyl-HSLs of bacteria by attaching to bacterial cells in marine biofilms [45].

Higher plants have also been shown to mount extensive responses to AHLs. Plants infected with the pathogen *E. carotovora* will respond by raising the pH at the site of infection [46]. Alkaline conditions cause the lactone ring to open, thereby preventing QS-controlled expression of virulence factors. The free-living terrestrial nematode *C. elegans* is attracted to AHLs and was shown to use AHLs for both aversive and attractive learning [47].

Likewise, bacteria can use signals produced by eukaryotes as cues. Another recently identified signal whose production is affected by a *luxS* mutation, although not produced by LuxS per se, is AI-3 [48], which is reported to be an aromatic compound without a sugar skeleton like AI-2. AI-3 is responsible for activation of transcription of the locus of enterocyte effacement (LEE) pathogenicity island genes and flagella genes in enterohaemorrhagic *E. coli* (EHEC) [49]. A *luxS* mutant was shown to be defective in expression of the LEE genes, but was unexpectedly still able to produce lesions on tissue culture cells. The authors identified epinephrine and norepinephrine as the signals that restored activation in the absence of AI-3. Thus, in the intestine, EHEC senses AI-3 produced by normal GI flora and epinephrine/norepinephrine produced by the host to activate expression of LEE genes. This AI-3/ epinephrine/norepinephrine signalling cascade is present in several bacterial species (*Shigella, Salmonella, Pasteurella, Haemophilus*) [48]. Indeed, both AI-2 and AI-3 activity have been detected in anaerobically cultured stools from healthy volunteers [49].

AHL QS molecules have also been shown to modulate gene expression of mammalian organisms. There have been numerous reports on the effect of QS signals on the host immune response [50–54]. The responses are many and varied, and there have been conflicting reports where AHLs act in moderating either inflammatory [55, 56] or humoral [54] immune T-cell responses. For example, 3-oxo-C12-HSL was found to increase the production of cyclooxygenase-2 and prostaglandin E_2 in human lung fibroblasts [56] as well as gamma interferon by T-cells, which led the authors to propose that AHLs were promoting a T-helper 1 phenotype. In contrast, Telford et al. [54] reported that interleukin-12 and tumour necrosis factor alpha production were inhibited in vitro by 3-oxo-C12-HSL, and subsequently proposed that this AHL shifted the T-cell-mediated immune response from a pro-inflammatory to an anti-inflammatory (Th-2) response. Ritchey et al. [52] suggested that this might in part reflect the underlying genetic bias of the host being tested in those experiments, and Hooi et al. [57] proposed that these differences could be attributed to high versus low doses of AHLs in these systems. It has also been shown that 3-oxo-C12-HSL is involved in repressing the oxidative burst of polymorphonuclear cells, which was proposed to protect biofilms of *P. aeruginosa* [58]. These authors were also able to show that QS antagonists had the opposite effect, and induced an oxidative burst in PMNs in vitro when added to *P. aeruginosa* biofilms. Thus, it is clear from these studies that the AHLs alter cytokine expression in eukaryotic hosts, and it has been suggested that the QS signal itself may constitute a virulence factor in its own right, modifying the host response in such a way as to enhance the ability of the invader to colonise the host.

QS manipulation

The effects of QS on the human immune system may be viewed as cues whereby the immune system is responding to the presence of the signal, but may also be viewed as chemical manipulation, where the bacterium is able to modulate the response of the immune system. A clear case of interference of signalling is the fact that human epithelial cells are also able to inactivate *P. aeruginosa* AHLs, indicating that humans have evolved QS interference strategies [59]. Differentiated human airway epithelial cells are able to inactivate 3-oxo-C12-HSL but not C4-HSL, indicating that the inactivation is selective for AHLs with certain acyl side chains. Epithelia exposed to environmental pathogens, such as lung and colon tissue, showed greater inactivation than those not normally exposed, such as kidney [59], indicating co-evolution of AHL-interference and AHL-producing microflora.

QS biomimics

Numerous examples of biomimicry exist in nature. The model legume *Medicago truncatula* can detect nanomolar to micromolar concentrations of AHLs and responds by producing over 150 proteins [60] as well as secreting QS signal mimics that potentially interfere with bacterial QS. Other plants have been shown to produce AHL mimics, possibly to manipulate the microbial rhizosphere population. Seedlings of various plant species and extracts from pea seedling exudate induces swarming in *Serratia liquefaciens* and activation of several AHL reporter systems, in addition to inhibiting QS-regulated phenotypes in *C. violaceum* [61].

One of the best examples of the production of QS antagonist by eukaryotes is the production of halogenated furanones by *Delisea pulchra*. This red marine alga produces a range of furanones (Fig. 1c) that are able to disrupt AHL signalling systems in many bacteria [62]. The secretion of these compounds onto the surface of the seaweed prevents the colonisation of the plant by bacteria [63], a phenotype that is QS-regulated in a number of

bacterial species. Furanones bind to and destabilise LuxR-family response regulator proteins [64]. Thus, these compounds have numerous applications where the inhibition of colonisation is relevant, for example, as antifouling treatments for ships or in biomaterials such as catheters and contact lenses. The fact that many bacterial pathogens regulate virulence by QS systems has led to the investigation of synthetic furanone derivatives as potential inhibitors of pathogenicity. Mortality of the shrimp *Penaeus monodon* was reduced to 50 % after intramuscular injection with diluted supernatant extracts from *V. harveyi* grown in the presence of furanones. It was shown that the production of an extracellular toxin produced by *V. harveyi* was inhibited by the furanones [65]. In addition, it has been demonstrated through the use of DNA microarrays that synthetic derivatives of furanones specifically target the QS regulon in *P. aeruginosa* and inhibit virulence factor production [66]. In a mouse pulmonary infection model, the furanones inhibited QS of *P. aeruginosa* in the mouse lung and promoted their clearance by the mouse immune response. Furthermore, treatment with furanones reduced the severity of lung pathology and prolonged survival time of the infected mice [67, 68]. These studies support the concept that furanones can be used as in vivo therapeutics to treat bacterial infections.

In addition to these interactions with the AHL QS system, furanones have been shown to specifically interact with the AI-2 signalling system. Microarray analysis revealed that 166 genes were differentially expressed in a *luxS* mutant of *E. coli* compared to the wild type and 90 genes were differentially expressed by addition of furanones [69]. Of the genes repressed by furanones, 79% were induced by AI-2. Furthermore, the furanones were shown to repress biofilm formation in *B. subtilis* and *E. coli*, a phenotype often regulated by QS systems [70]. We have shown that synthetic furanones affect QS-regulated virulence factor expression in *Vibrio vulnificus* and *Vibrio cholerae* (McDougald et al., unpublished). The addition of furanones inhibited metalloprotease and hemolysin expression in a dose-dependent manner. Further to this, we have shown that pretreatment of *V. vulnificus* with furanones prevents virulence in a mouse model of infection.

Chlamydomonas reinhardtii, a unicellular green alga, produces both agonists and antagonists of *P. aeruginosa* QS. Ethyl acetate extracts of *C. reinhardtii* cultures contained more than a dozen chemically separable but unidentified AHL mimics that stimulated LuxR homologues [71]. In addition, colonies of *C. reinhardtii* and *Chlorella* sp. stimulated luminescence in the *V. harveyi* AI-2 reporter strain. Colonies of *C. reinhardtii* also inhibited QS-regulated luminescence in a LasR reporter, indicating the production of both agonist and antagonist compounds by this alga.

In a screen of natural and synthetic compound libraries, extract of garlic was found to act as a potent inhibitor of QS-regulated genes, such as alginate and elastase, in *P. aeruginosa* [72]. Addition of garlic extract to *P. aeruginosa* biofilms resulted in a flat, undifferentiated biofilm, much like that formed by the QS mutant. Co-treatment of *P. aeruginosa* with garlic extract and tobramycin resulted in more effective killing than in biofilms treated with tobramycin only. Furthermore, the garlic extract was effective in vivo, where the virulence of *P. aeruginosa* was reduced to 40% in a *C. elegans* model. Screening of *Penicillium* spp. revealed that 66% produced secondary metabolites with QS inhibitory activity, and that these compounds accelerated the turnover of LuxR [72].

Thus, it is clear that QS antagonists have evolved in a broad range of hosts that interact with bacteria. This indicates that there is a large, untapped resource of QS inhibitors, and that QS-mediated community-based phenotypes of bacteria have existed long enough for hosts to evolve chemistries that interfere with these interactions. One very exciting area of research will be to identify QS receptors and pathways in these eukaryotes in order to better understand the interaction of the two kingdoms (see [73] for a recent review on interkingdom signalling).

Rational design of inhibitors

The use of QS inhibitors that are biomimics of natural interference systems is a rational approach to the development of biocontrol strategies. The goal of such studies is to learn how organisms naturally disrupt QS, and to then identify the key features of those disrupting techniques to subsequently manipulate them into better inhibitors through either synthetic chemistry or gene technology in order to artificially express, for example, AHL degrading enzymes in important crop species. Because many pathogens require QS to produce virulence factors in response to association with human hosts, QS is a target for the design of small-molecule inhibitors.

Investigations of AHL analogues demonstrated that the homoserine lactone group was important for AI function and the ring must be small for binding to the receptor, while the acyl chain was less important [74]. Further studies on acyl chain analogues demonstrated that the length of the acyl chain can be altered to some degree without abolishing activity, although dramatic changes eliminate activity [74–76]. These studies also demonstrated that changes at the 3 position eliminate activity and the acyl chain geometry must be maintained. Zhu et al. [77] tested 33 analogues of 3-oxo-C8-HSL and found that only those that differ in length or level of desaturation of the acyl chain were weakly active, but at least half of the analogues

functioned as antagonists. Another study tested 22 novel AHLs with substituents at the C4 position and determined that alkyl analogues were mostly agonists, while aryl analogues were antagonists [78].

These types of studies can be combined with molecular modelling as an attractive approach to targeted drug design. Identification of the receptor pocket and determination of ligand interactions could lead to the subsequent generation of optimal inhibitors for receptor binding. Obtaining the LuxR crystal structure has been difficult, presumably because these receptor proteins are membrane-bound. However, the crystal structure of TraR has been solved with the signal bound in the pocket. Using historical data on AHL activities in TraR bioassays, Goh et al. [79] showed correlations between docking scores of AHLs and their activity, suggesting that molecular modelling may also be used to generate novel inhibitors of QS systems.

The use of AHL degradation as a means of control has been tested in a number of systems. For example, transgenic plants expressing AiiA are resistant to QS-dependent bacterial infection by *E. carotovora* [28]. As mentioned previously, early expression of AHL-regulated virulence factors may result in clearance of the pathogen. This has been shown to occur in vivo, where transgenic tobacco plants expressing AHL synthase from *E. carotovora* were more resistant to infection by *E. carotovora* than the wild type plant [80]. Similarly, addition of native AHLs to tobacco plants inoculated with *E. carotovora* decreased disease to 10%, providing important proof-of-principle that interaction between bacteria and their eukaryotic hosts can be manipulated by disrupting QS signalling.

The fact that QS systems have evolved numerous times in the history of prokaryotes indicates that there must be some selective advantage awarded to bacteria that are able to communicate via QS systems. There is growing evidence that eukaryotes have an extensive range of functional responses to AHLs that may play important roles in the beneficial or pathogenic outcomes of eukaryote–prokaryote interactions. Thus, the rational design of biomimics that are able to interfere with bacterial QS systems is a promising means of manipulating bacterial populations.

Conclusions

It is clear that QS has evolved as a mechanism for organisms to probe the local environment and to coordinate behaviour and response for competitive advantages. Not surprisingly, this has led to the evolution of a variety of interactions, ranging from symbiotic to antagonistic, and these can involve the manipulation of one species by another. The origins and evolutions of these interactions are quite intriguing and will hopefully lead to the rational design of manipulation technologies for novel bacterial control strategies.

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References

1. Beck von Bodman S, Majerczak DR, Coplin DL (1998) Proc Natl Acad Sci USA 95:7687–7692
2. Eberhard A, Burlingame AL, Eberhard A, Kenyon GL, Nealson KH, Openheimer JJ (1981) Biochem 20:2444–2449
3. Nealson KH, Markovitz A (1970) J Bacteriol 104:300–312
4. Fuqua WC, Winans SC, Greenberg PE (1994) J Bacteriol 176:269–275
5. Whiteley M, Lee KM, Greenberg EP (1999) Proc Natl Acad Sci USA 96:13904–13909
6. Rumbaugh KP, Griswold JA, Hamood AN (2000) Microbes Infect 2:1721–1731
7. Chugani SA, Whiteley M, Lee KM, D'Argenio D, Manoil C, Greenberg EP (2001) Proc Natl Acad Sci USA 98:2752–2757
8. Lequette Y, Lee J-H, Ledgham F, Lazdunski A, Greenberg EP (2006) J Bacteriol 188:3365–3370
9. Ledgham F, Ventre I, Soscia C, Foglino M, Sturgis JN, Lazdunski A (2003) Mol Microbiol 48:199–210
10. Lee J-H, Lequette Y, Greenberg EP (2006) Mol Microbiol 59:602–609
11. Minogue TD, Wehland-von Trebra M, Bernhard F, von Bodman SB (2002) Mol Microbiol 44:1625–1635
12. Zhu J, Winans SC (2001) Proc Natl Acad Sci USA 98:1507–1512
13. Vannini A, Volpari C, Gargioli C, Muraglia E, Cortese R, De Francesco R, Neddermann P, Di Marco S (2002) EMBO J 21:4393–4401
14. Medina G, Juarez K, Valderrama B, Soberon-Chavez G (2003) J Bacteriol 185:5976–5983
15. Van Houdt R, Aertsen A, Moons P, Vanoirbeek K, Michiels CW (2006) FEMS Microbiol Lett 256:83–89
16. Riedel K, Hentzer M, Geisenberger O, Huber B, Steidle A, Wu H, Hoiby N, Givskov M, Molin S, Eberl L (2001) Microbiology 147:3249–3262
17. Bassler BL, Wright M, Showalter RE, Silverman MR (1993) Mol Microbiol 9:773–786
18. Bassler BL, Wright M, Silverman MR (1994) Mol Microbiol 13:273–286
19. Surette MG, Miller MB, Bassler BL (1999) Proc Natl Acad Sci USA 96:1639–1644
20. Lombardia E, Rovetto AJ, Arabolaza AL, Grau RR (2006) J Bacteriol 188:4442–4452
21. Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM (2002) Nature 415:545–549
22. Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM (2004) Mol Cell 15:677–687
23. Taga ME, Miller ST, Bassler BL (2003) Mol Microbiol 50:1411–1427
24. Taga ME, Semmelhack JL, Bassler BL (2001) Mol Microbiol 42:777–793
25. Xavier KB, Bassler BL (2005) J Bacteriol 187:238–248
26. Winzer K, Hardie KR, Burgess N, Doherty N, Kirke D, Holden MTG, Linforth R, Cornell KA, Taylor AJ, Hill PJ, Williams P (2002) Microbiology 148:909–922

27. Doherty N, Holden MTG, Qazi SN, Williams P, Winzer K (2006) J Bacteriol 188:2885–2897
28. Dong Y-H, Wang L-H, Xu J-L, Zhang H-B, Zhang X-F, Zhang L-H (2001) Nature 411:813-817
29. Lee SJ, Park S-Y, Lee J-J, Yum D-Y, Koo B-T, Lee J-K (2002) Appl Environ Microbiol 68:3919–3924
30. Reimmann C, Ginet N, Michel L, Keel C, Michaux P, Krishnapillai V, Zala M, Heurlier K, Triandafillu K, Harms H, Defago G, Haas D (2002) Microbiology 148:923–932
31. Leadbetter JR, Greenberg EP (2000) J Bacteriol 182:6921–6926
32. Uroz S, D'Angelo-Picard C, Carlier A, Elasri M, Sicot C, Petit A, Oger P, Faure D, Dessaux Y (2003) Microbiology 149:1981–1989
33. Carlier A, Uroz S, Smadja B, Fray R, Latour X, Dessaux Y, Faure D (2003) Appl Environ Microbiol 69:4989–4993
34. Huang JJ, Han JI, Zhang LH, Leadbetter JR (2003) Appl Environ Microbiol 69:5941–5949
35. Park SY, Lee SJ, Oh TK, Oh JW, Koo BT, Yum DY, Lee JK (2003) Microbiology 149:1541–1550
36. Lin Y-H, Xu J-L, Hu J, Wang L-H, Ong SL, Leadbetter JR, Zhang L-H (2003) Mol Microbiol 47:849–860
37. Park S-Y, Kang H-O, Jang H-S, Lee J-K, Koo B-T, Yum D-Y (2005) Appl Environ Microbiol 71:2632–2641
38. Sio CF, Otten LG, Cool RH, Diggle SP, Braun PG, Bos R, Daykin M, Camara M, Williams P, Quax WJ (2006) Infect Immun 74:1673–1682
39. Visick KL, Foster J, Doino J, McFall-Ngai M, Ruby EG (2000) J Bacteriol 182:4578–4586
40. Lupp C, Ruby EG (2005) J Bacteriol 187:3620–3629
41. Schaefer AL, Wier AM, Graber JR, DeLoney-Marino CR, McFall-Ngai MJ, Ruby EG (2005) Coming out of the dark: using genomics to shed light on the squid-*Vibrio* symbiosis. Presented at VIBRIO 2005, 7–8 November 2005, Ghent, Belgium
42. Nishiguchi MK, Ruby EG, McFall-Ngai MJ (1998) Appl Environ Microbiol 64:3209–3213
43. Visick KL, Fuqua C (2005) J Bacteriol 187:5507–5519
44. Tait K, Joint I, Daykin M, Milton DL, Williams P, Camara M (2005) Environ Microbiol 7:229–240
45. Joint I, Tait K, Callow ME, Callow JA, Milton D, Williams P, Camara M (2002) Science 298:1207
46. Nachin L, Barras F (2000) Mol Plant Microbe Interact 13:882–886
47. Beale E, Li G, Tan M-W, Rumbaugh KP (2006) Appl Environ Microbiol 72:5135–5137
48. Reading NC, Sperandio V (2006) FEMS Microbiol Lett 254:1–11
49. Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB (2003) Proc Natl Acad Sci USA 100:8951–8956
50. DiMango E, Zar HJ, Bryan R, Prince A (1995) J Clin Invest 96:2204–2210
51. Li L, Hooi D, Chhabra SR, Pritchard D, Shaw PE (2004) Oncogene 23:4894–4902
52. Ritchie AJ, Yam AOW, Tanabe KM, Rice SA, Cooley MA (2003) Infect Immun 71:4421–4431
53. Tateda K, Ishii Y, Horikawa M, Matsumoto T, Miyairi S, Pechere JC, Standiford TJ, Ishiguro M, Yamaguchi K (2003) Infect Immun 71:5785–5793
54. Telford G, Wheeler D, Williams P, Tomkins PT, Appleby P, Sewell H, Stewart GSAB, Bycroft BW, Pritchard DI (1998) Infect Immun 66:36–42
55. Smith RS, Harris SG, Phipps R, Iglewski B (2002) J Bacteriol 184:1132–1139
56. Smith RS, Kelly R, Iglewski B, Phipps R (2002) J Immunol 169:2636–2642
57. Hooi DSW, Bycroft BW, Chhabra SR, Williams P, Pritchard DI (2004) Infect Immun 72:6463–6470
58. Bjarnsholt T, Jensen PO, Burmolle M, Hentzer M, Haagensen JAJ, Hougen HP, Calum H, Madsen KG, Moser C, Molin S, Hoiby N, Givskov M (2005) Microbiology 151:373–383
59. Chun CK, Ozer EA, Welsh MJ, Zabner J, Greenberg EP (2004) Proc Natl Acad Sci USA 101:3587–3590
60. Mathesius U, Mulders S, Gao M, Teplitski M, Caetano-Anolles G, Rolfe BG, Bauer WD (2003) Proc Natl Acad Sci USA 100:1444–1449
61. Teplitski M, Robinson JB, Bauer WD (2000) Mol Plant Microbe Interact 13:637–648
62. Givskov M, De Nys R, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S (1996) J Bacteriol 178:6618–6622
63. Kjelleberg S, Steinberg P, Givskov M, Gram L, Manefield M, de Nys R (1997) Aquat Microb Ecol 13:85–93
64. Manefield M, Rasmussen TB, Henzter M, Andersen JB, Steinberg P, Kjelleberg S, Givskov M (2002) Microbiology 148:1119–1127
65. Manefield M, Harris L, Rice SA, De Nys R, Kjelleberg S (2000) Appl Environ Microbiol 66:2079–2084
66. Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song ZJ, Kristoffersen P, Manefield M, Costerton JW, Molin S, Eberl L, Steinberg P, Kjelleberg S, Hoiby N, Givskov M (2003) EMBO J 22:3803–3815
67. Wu H, Song Z, Givskov M, Doring G, Worlitzsch D, Mathee K, Rygaard J, Høiby N (2001) Microbiology 147:1105–1113
68. Wu H, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, Hoiby N (2004) J Antimicrob Chemother 53:1054–1061
69. Ren D, Bedzyk LA, Ye RW, Thomas SM, Wood TK (2004) Biotech Bioeng 88:630–642
70. Ren DC, Sims JJ, Wood TK (2001) Environ Microbiol 3:731–736
71. Teplitski M, Chen HC, Rajamani S, Gao MS, Merighi M, Sayre RT, Robinson JB, Rolfe BG, Bauer WD (2004) Plant Physiol 134:137–146
72. Rasmussen TB, Bjarnsholt T, Skindersoe ME, Hentzer M, Kristoffersen P, Kote M, Nielsen J, Eberl L, Givskov M (2005) J Bacteriol 187:1799–1814
73. Shiner EK, Rumbaugh KP, Williams SC (2005) FEMS Microbiol Rev 29:935–947
74. Eberhard A, Widrig CA, McBath P, Schineller JB (1986) Arch Microbiol 146:35–40
75. Passador L, Tucker KD, Guertin KR, Journet MP, Kende AS, Iglewski BH (1996) J Bacteriol 178:5995–6000
76. Schaefer AL, Hanzelka BL, Eberhard A, Greenberg EP (1996) J Bacteriol 178:2897–2901
77. Zhu J, Beaber JW, Moré MI, Fuqua C, Eberhard A, Winans SC (1998) J Bacteriol 180:5398–5405
78. Reverchon S, Chantegrel B, Deshayes C, Doutheau A, Cotte-Pattat N (2002) Bioorg Med Chem Lett 12:1153–1157
79. Goh W, Rice SA, Kumar N (2005) Molecules 10:1263–1271
80. Mae A, Montesano M, Koiv V, Palva ET (2001) Mol Plant Microbe Interact 14:1035–1042
81. Camilli A, Bassler BL (2006) Science 311:1113–1116