Microbial Communication via Quorum Sensing

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(Invited Paper)

***Abstract*—Chemical communication enables microbes to probe local cell density and coordinate collective behavior through a process known as quorum sensing (QS). In QS, microbes produce and detect small molecule signals, and the expression levels of many genes change in response to these signals. QS signals, known as autoinducers, potentially accumulate around the cell and relay information about environmental conditions, transport dynamics, and the number and identity of microbial neighbors. In this paper, we focus on the history of QS, the variety of molecular networks used by microbes to achieve QS, modeling approaches, and applications of QS control.**

***Index Terms*—Molecular communication, biological interactions, biological system modeling, synthetic biology, bioluminescence, cells.**

I. Introduction

MICROBES may be small, but their impact on our everyday lives is enormous. Working together, communities of microorganisms are critical in processes such as environmental remediation, wastewater treatment, and human health and disease, and there is great interest in designing synthetic microbial systems as sensors and for biosynthesis of fuels, drugs, and chemical feedstocks [1]–[3]. At the core of many of these important microbial functions is chemical communication. The exchange of small molecule signals within microbial populations, generally referred to as *quorum sensing*, is ubiquitous in nature and enables cells to respond to fluctuations in cell density and activity by specific coordinated behavior. In Table I, we list a few noted microbes that utilize quorum sensing to control behaviors such as virulence, bioluminescence, the formation of surfaced associated microbial communities called *biofilms*, and the production of antibiotics and other bioactive compounds [4]–[6]. There have been many excellent reviews on quorum sensing over the years [4], [7]–[11]. Here we focus particularly on the history of quorum sensing, its applications, the variety of molecular architectures microbes implement to communicate, and the theoretical approaches that have been applied to understand these signaling networks.

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II. The Discovery of Quorum Sensing

Quorum sensing (QS) or *autoinduction* as it was originally called, was discovered by searching for an explanation for one of those "it doesn't fit the model" phenomena – namely the pattern of development of the bioluminescence (lux) system during growth of luminous bacteria [20]. At the time, the 1965 Nobel Prize had recently been awarded for studies on the mechanism of enzyme induction and repression, and the concept that some genes were expressed and regulated in groups called *operons*. One such operon involved the emission of light from dense populations of the marine organism *Vibrio harveyi*. Fig. 1 shows the intensity of light emission (and luciferase synthesis) during the growth of *V. harveyi*: this pattern, which is common in most luminous bacteria that have been examined [22], [23], shows a period of no increase in luminescence followed by a burst of synthesis at a rate much greater than the rate of growth [20]. So what didn't fit here? The paradigm of the day (gene induction/repression) involved the ability of bacteria to sense their environment, and respond to added compounds by the synthesis or repression of enzymes at the level of transcription. To this end, *V. harveyi* appeared to behave like an inducible system, but without the addition of an inducer: hence the name of autoinduction [20]. These discoveries mirrored reports of autoinduction in control of competence, or the ability of some microbes to uptake DNA from the environment. In cultures of *Pneumococcus* the activation of competence was shown to be induced by compounds released by microbes into the growth media [24].

In the case of *V. harveyi*, cells seemed to transcribe a gene that "turned on" light production. This autoinducer seemed to be released in the growth media, as cells diluted into fresh medium ceased light production and transferring cells to "used" medium led to an early induction of the *lux* system [20]. Whatever was released by the cells was very unstable, disappearing in hours in conditioned medium, and could be destroyed upon boiling (KHN, personal communication) [25], [26]. During this stage of work, although hundreds of dim and dark mutants were isolated [25], not one was shown to be defective in the production of AI activity, an observation that would be explained many years later, when it was revealed that the QS system controlled many genes simultaneously (see below), many of which were

TABLE I
WELL-STUDIED BACTERIAL QUORUM SENSING SYSTEMS AND KEY REGULATED FUNCTIONS [7], [12]–[19]

Organism	Environment	Quorum sensing signal(s)	Key regulated function
Bacillus subtilis	soil	modified peptide chain	DNA uptake
Chromobacterium violaceum	soil	homoserine lactone	pigment production
Pseudomonas aeruginosa	soil and fresh water, human pathogen	homoserine lactones, quinolone signal, IQS	virulence, biofilm formation, enzyme production
Erwinia carotovora	soil, plant pathogen	homoserine lactone, autoinducer-2	plant virulence, antibiotic production
Staphylococcus aureus	human body	modified peptide chain	virulence, biofilm formation
Vibrio cholerae	marine, pathogen	autoinducer-2, CAI-1	virulence (causative agent of cholera), biofilm formation
Vibrio fischeri	marine	homoserine lactones, autoinducer-2	Bioluminescence (light production in squid)

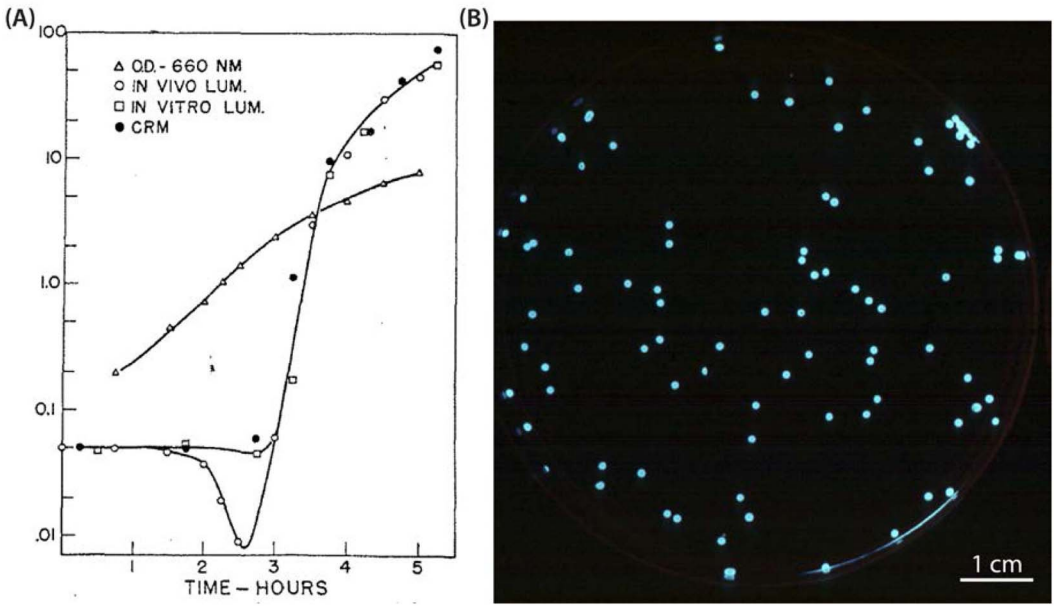

Fig. 1. Growth, luminescence, luciferase (in vitro luminescence), and cross reacting material (CRM) to luciferase antibody as a function of time [20]. Photograph taken using the light produced by bioluminescent colonies of the marine bacterium *Vibrio fischeri*.

important for growth and survival. Although activity could be seen in related *Vibrio fischeri*, it was virtually impossible to get a reproducible cell-based assay of autoinducer activity. More than a decade later, a series of key experiments were conducted to decipher the mechanism of autoinduction.

A major advance in the understanding of autoinduction occurred via the isolation of two strains of luminous bacteria – strain B-61, isolated by Dr. P. Baumann was identified as a non-luminous strain of *V. fischeri*, and strain MJ-1, (isolated by KHN from the light organ of a bioluminescent fish, *Monocentris japonica),* identified as a hyper-luminous strain of *V. fischeri* [27], [28]. Strain B-61, while dark to the human eye, turned out to be very dim, a state that was due to the lack of production of the activator compound (AI) – this strain provided for the first time, a method for quantitative assay for AI [29]. Meanwhile, MJ-1 was so bright because it produced very high levels of AI activity, and was the source of AI for purification and identification. Purification and identification of the first AI was achieved, revealing a heretofore unknown biological activator: the first acyl-homoserine lactone [30], [31], as shown in Fig. 2. A personal note here: at the time of these experiments, the concept of autoinduction was not a popular one, and the careful work of Anatol Eberhard resulted in not only the identification of the AI, but its synthesis (and the synthesis of several inactive chemical variants). The availability of synthetic AI that exhibited full activity was an important advance in convincing "the doubters" that autoinduction was real and that the homoserine lactone acted at the level of transcription, inducing the synthesis of the luminous system [29]–[31].

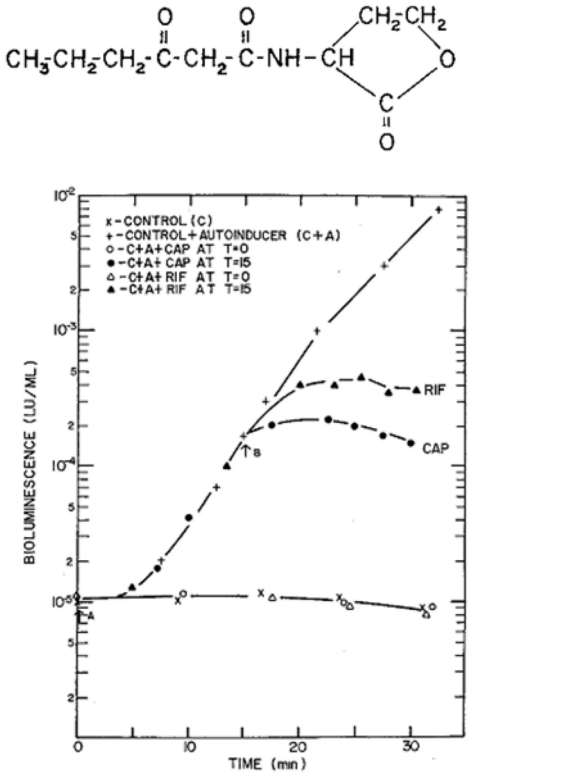

Fig. 2. Structure of the autoinducer molecule (AI), and the effect of AI addition with and without inhibitors of protein synthesis (chloramphenicol, CAP) or mRNA synthesis (rifampin, RIF), demonstrating that its activity is at the level of transcription [29].

One question that remained was how the AI could be produced in the cell and not immediately induce the lux system. To this end, Rosson and Nealson [22] demonstrated that at very low cell density, luminescence synthesis would cease, and that this could be reversed by the addition of pure AI back to the chemostat culture. Greenberg *et al.* [32] subsequently showed that the AI in *V. harveyi* was completely membrane permeable. The induction of bioluminescence required the slow accumulation of the autoinducer in the medium. The cloning and functional identification of the genes controlling autoinduction (*luxI* and *luxR*), and their expression in *E. coli* [16] represented a major change in the development of the field: it was the first time that a model of autoinduction was allowed in print, and led to broad acceptance of the process that was to be called *quorum sensing:* one might say it was "grudgingly accepted" at this point.

The first published "model" of quorum sensing (16), provided an explanation for the process, as shown in Fig. 3. While the details of the mechanism have advanced to remarkable levels of complexity, and variations on this theme have grown immensely over the years, the basic strategy for building and controlling a cell-density-dependent sensing mechanism has remained. The organization of *the lux* operon also answered one of the enigmatic questions that had plagued early investigators: why was the bioassay so non-linear? As can be seen in Fig. 3, the system in *V. fischeri* is designed so that when it is induced, the first product synthesized is the enzyme that makes the autoinducer (AI); i.e., it is a feed-forward, nonlinear induction system: who would have thought? Similar quorum sensing system would later be found in numerous species of bacteria, the details of which are described below.

The rationale for quorum sensing in *Vibrio* species related directly to the life-style of the luminous bacteria [23]. These bacteria are found in high concentration on decaying animal material, in the stomachs of many marine fishes that eat such material, and in the light organs of some luminous fishes and squids. However they are also found at low cell densities as free floating (planktonic) forms in seawater [33], [34]. There is compelling evidence to show that marine fish are attracted to the bright particles [35], providing a mechanism for returning these "gut bacteria" to a nutrient-rich protected niche (until they are again unceremoniously excreted into the seawater environment). Given that the emission of light in fully induced luminous bacteria is energetically expensive, autoinduction provides a simple and effective mechanism for turning off the light when these bacteria are floating free in the ocean water at low cell densities [23], [29].

III. Is Quorum Sensing Common?

Quorum sensing appears to be a ubiquitous approach that nearly all unicellular living systems implement to respond to a variety of situations in which monitoring the local population density would be beneficial [36]. The ability to produce and detect the same molecular signal leads to coordination of behavior within large groups of cells, potentially increasing the efficiency of processes that require a large population of cells working together. The release and recapture of a molecular signal also probes the transport properties of the local environment [37], [38]. Cells can use this information to determine whether or not the release of more costly cellular products such as enzymes is a feasible strategy [39]. The positive feedback can even lead to differentiation of cellular functions within a population [36], potentially enabling division of labor and more complex "social" behaviors within populations [9], [40].

Quorum sensing is a ubiquitous mechanism of gene regulation in bacteria. There are at least 70 strains of bacteria with characterized quorum sensing mechanisms [41], and the list continues to grow [42]. Within the well-characterized quorum sensing pathways, there are several different "wiring diagrams" that cells use to achieve density dependent gene regulation [7]. Here, we discuss some core architectures of quorum sensing systems that have been identified in a variety of bacterial species over the past forty years, and discuss some of the models that have been used to quantitatively understand the potential outputs and properties of these self-signaling networks.

IV. A Basic Quorum Sensing System

At its core, a quorum sensing system is composed of a signal producing enzyme and a signal receptor. Many of the quorum sensing systems that seemed simple, such as the initial networks from *Vibrio* species described above [16], are now known to involve multiple signals or layers of regulatory feedback [43]. One system that implements a more basic model of quorum sensing is the N-acylhomoserine lactone system

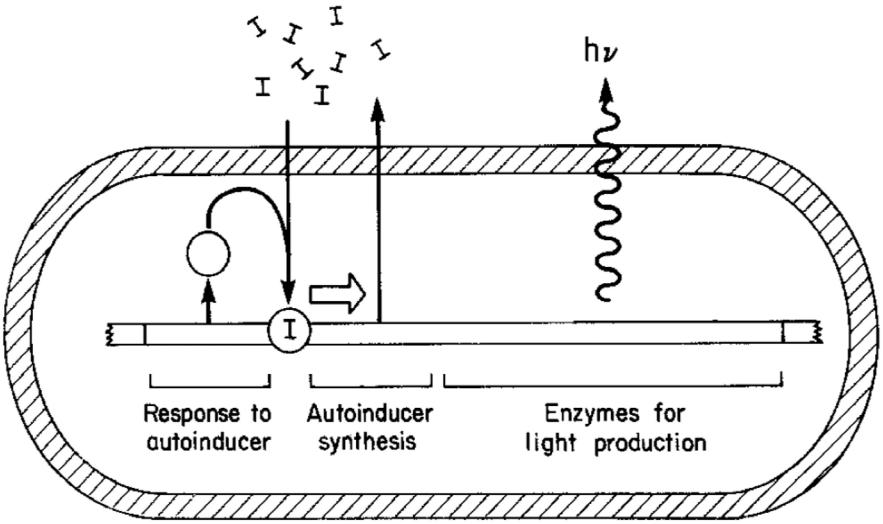

Fig. 3. Model of autoinduction first presented by Engebrecht *et al.* The response to autoinducer is encoded in the gene luxR that, in combination with AI, turns on the lux operon; autoinducer synthesis is encoded in the luxI gene that catalyzes the synthesis of AI; enzymes for light production include the luxAB genes that code for the two subunits of bacterial luciferase and the luxCDE genes that code for proteins that catalyze the synthesis of a long chain aldehyde needed for light emission. Reproduced from [16].

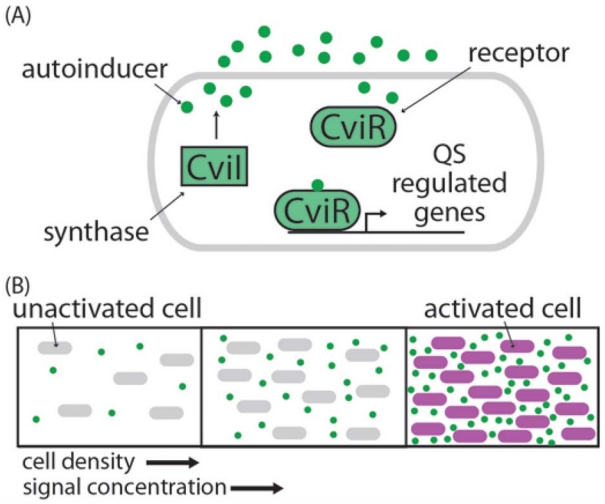

Fig. 4. A) The basic quorum sensing system, such as CviI/CviR in *Chromobacterium violaceum*, includes a synthase that makes the autoinducer and a receptor that binds the autoinducer. A bound receptor is activated to regulate the expression of quorum sensing controlled genes. B) As cell density increases, the concentration of the autoinducer increases. When the autoinducer concentration exceeds a threshold, receptors become activated and the cells in the population express quorum sensing regulated genes.

CviI/CviR found in *Chromobacterium violaceum*, depicted in Fig. 4. The *cviI* gene encodes a cytosolic enzyme that produces a specific acylated homoserine lactone. The autoinducer signal produced by *cviI* in the strain 12472 is N-decanoyl-L-homoserine lactone [13], also referred to as C10-HSL or DHL. Once synthesized, the signal diffuses into and out of the cell and potentially accumulates in the vicinity of the cell. The receptor for C10-HSL is the CviR protein [13]. In many cases the signal producing enzyme and the receptor are co-transcribed from the same promoter, although this is not the case for CviI/CviR [13]. Binding of the autoinducer to the receptor often forms a receptor dimer that is more stable than the unbound receptor [44]. The affinity for a typical autoinducer to its receptor is about 10 nM [45]. There is some evidence that signal-induced dimerization increases receptor stability, specifically for TraR and QscR [44], [46], although it is yet unknown if this is typical of HSL binding receptors. In addition, the receptor can now act as a transcriptional regulator, specifically binding throughout the genome to an inverted palindrome binding site [13]. Once bound to this recognition sequence upstream of a gene, the autoinducer-receptor complex will increase the rate of mRNA production of the adjacent gene [13].

In many species, quorum sensing systems are strong regulators of global gene expressions. In *C. violaceum*, CviR regulates more than 20 genes [13]. In other species such as *Pseudomonas aeruginosa*, 6% of the genome or over 300 genes, are regulated by quorum sensing [14]. As a transcriptional regulator, the signal-bound receptor participates in regulatory decisions throughout the genome, helping to determine which genes to transcribe into mRNA and ultimately influencing protein concentrations within the cell. *C. violaceum* has a basic quorum sensing system, involving a single small molecule signal which exclusively binds to a single receptor.

Another relatively simple quorum sensing system is common in Gram-positive bacteria. Many Gram-positive bacteria, including *Streptococcus pneumoniae*, *Bacillus subtilis*, and *Staphylococcus aureus* use auto-inducing cyclic peptides (AIPs) as a quorum sensing signal [18], [47]. Given their cell wall/membrane structures, it isn't surprising that Gram-positive bacteria have settled on what appears to be, at first sight, a very different mode of cell-to-cell communication from that seen in the Gram-negatives. In the end, though, the basic idea persists, an autoinducer, which in this case is a peptide, termed an AIP accumulates in the growth medium and leads to a quorum-like response [7]. The AIP is secreted into the growth medium and acts either from the exterior, or in some cases, is modified and is taken up into the cell to

act intracellulary. In contrast to the HSL autoinducers which rapidly partition across the membrane by diffusion [48], the oligo-peptides, which are the result of post-translational cleavage of other proteins, must be actively transported across the membrane [49]. Detection of the AIPs is done by two-component (sensor kinase) detection mechanisms that respond to the peptide AIs, phosphorylating a regulatory protein inside the cell and ultimately initiating a response in the form of gene activation. As is beautifully documented in the Rutherford and Bassler review [18], almost any variation on this theme will probably exist. The key components for Gram-positive quorum sensing are: 1) the AI synthase; 2) the transporters to move the AIP out of (and back into) the cell; 3) the sensor/kinase systems to phosphorylate activator molecules; and 4) the sensitive promoters throughout the cell that ultimately respond to these systems. With these building blocks in hand, it is easy to build the complexities that are now being seen in the Gram-positive world of quorum sensing. As we will see in the subsequent examples, some cells use more complex quorum sensing systems that utilize multiple signals or receptors, or may even be involved in interspecies signaling.

V. Signal Integration in Vibrio harveyi

Some quorum sensing systems have the ability to produce and detect multiple signals, such as the quorum sensing systems of *V. harveyi*. *V. harveyi* produces three different signals known as CAI-1, HAI-1, and AI-2 [50], as shown in Fig. 5. These three signals act differently as compared to the quorum sensing systems of *C. violaceum* discussed previously in that the receptors are not loose within the cytoplasm, but instead are anchored to the inner membrane of the cell. When the autoinducer binds to and activates (or sometimes deactivates) a membrane-bound receptor, the receptor then transmits this change in state to an internal protein called a response regulator. The response regulator then acts to modulate gene expression within the cell. This setup of receptor and response regulator is called a two-component system [51]. In the case of *V. harveyi* signaling, the three signals, CAI-1, HAI-1, and AI-2, all bind to unique receptors that activate a common final response regulator, LuxR [52]. LuxR from *V. harveyi* does not directly bind to autoinducer and functions differently than the identically named LuxR from *V. fischeri* described earlier. The signal from each sensor protein is transmitted and processed through several intermediate steps involving the proteins LuxU and LuxO and regulatory small RNAs [50]. Signal transduction with multiple sensor kinases and/or response regulators like the one here is termed a phosphorelay [53].

By integrating the input from the three external signals, *V. harveyi* is able to activate multiple quorum sensing responses depending on the signaling environment [54], [55]. For example, both AI-2 and CAI-1 are produced by bacteria other than *V. harveyi*, enabling *V. harveyi* to integrate information about the local population density of itself and others [55]. The range of affinities for the response regulator to its binding targets throughout the genome enables different outputs states of the system [55]. Each gene directly regulated by the LuxR response regulator contains a specific

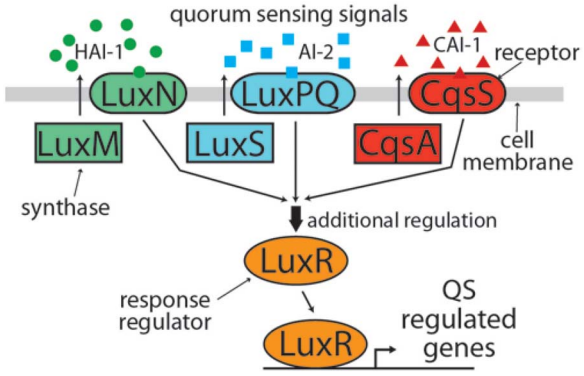

Fig. 5. *Vibrio harveyi* combines three different signals to control quorum sensing activation [50]. The receptors, embedded in the cell membrane, each bind a different autoinducer and regulate activity of a response regulator inside the cell. The response regulator controls expression of quorum sensing controlled genes. The thick arrow pointing towards LuxR represents additional steps involved in LuxR regulation, see [50].

DNA sequence that attracts the regulator, however not all genes have exactly the same binding sequence. Changes in the DNA binding sequence modulate the affinity for LuxR to the DNA, which contributes to differences in expression of each regulated gene. The titration of LuxR amongst its binding sites couples the distribution of binding site strengths to the set of genes regulated at low and high levels of LuxR activation.

VI. Hierarchical Signaling Cascades in Pseudomonas aeruginosa

Another quorum sensing system involving multiple signals working together can be found in *P. aeruginosa*. Four different and overlapping signaling systems work together to regulate density dependent behavior in *P. aeruginosa* [56], [57]. The LasI/LasR and the RhlI/RhlR both use homoserine lactone signals, similar to the CviI/CviR systems found in *C. violaceum*. The signals of these systems partition into the cell and dimerize the receptor to make it an active transcriptional regulator. Two other signals produced in *P. aeruginosa* are PQS (Pseudomonas quinolone signal) [58], [59] and IQS (2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde) [60], binding to and activating the receptors PqsR and IqsR, respectively.

Each signaling system activates its own transcriptional regulator instead of funneling signaling information into a common regulator as in *V. harveyi*. However these four quorum sensing networks are interconnected through transcriptional regulation, with the levels of synthases and receptors for each signal either regulating or being regulated by at least one other quorum sensing system [56], as shown in Fig. 6. The network is considered hierarchical, in the sense that LasI/LasR positively regulates the 3 other systems. Both positive and negative interactions directly among the four systems determine the changes in gene expression as the cell density increases. Indirect interactions, in which one of the quorum sensing systems induces production of additional transcription factors, participate in the regulation of other quorum sensing systems [56], [61]. In one example shown as part of Fig. 6C, the expression of the VqsR protein is increased, or upregulated, by LasI/LasR, VqsR

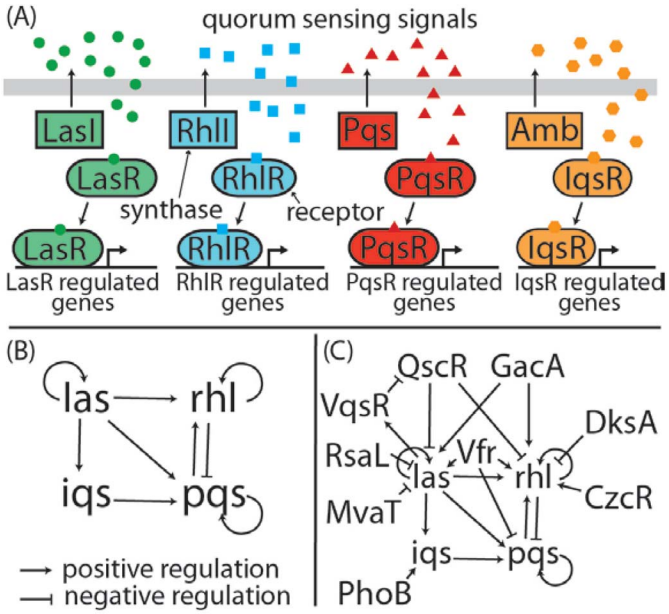

Fig. 6. A) *Pseudomonas aeruginosa* uses four different quorum sensing systems. Each system uses an independent signal and regulates a different set of genes, although some gene targets are regulated by multiple signals. B) The four quorum sensing systems positively and negatively regulate each other. C) Feedback between the four QS systems is part of a larger, interconnected network that regulates gene expression. Only a subset of known regulatory connections are shown, for further details (see [56], [63]–[65]).

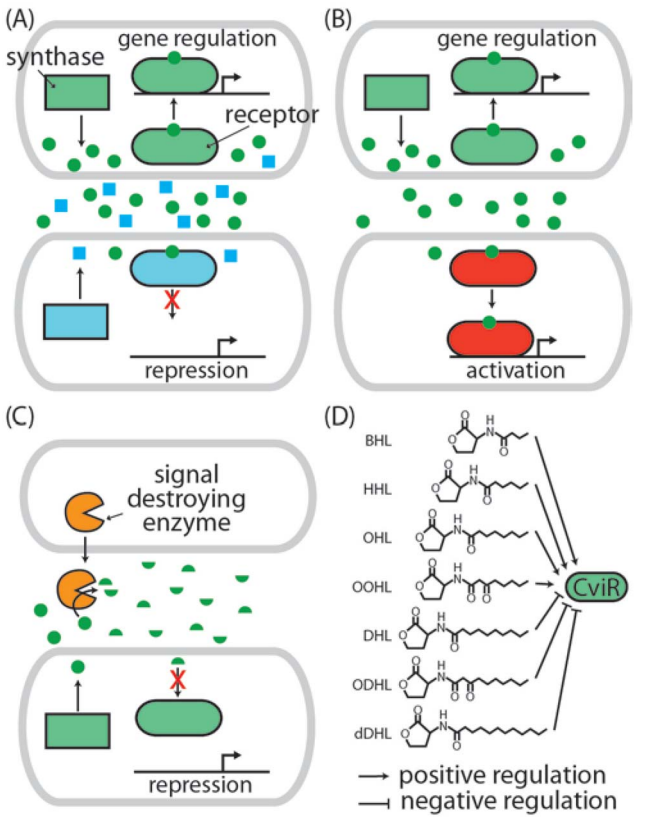

Fig. 7. Quorum sensing interactions between species. A) Signals from one strain can bind to the receptor of another strain potentially inhibiting quorum sensing activation. B) Strains that contain a receptor and no synthase require signal from a neighboring strain to activate quorum sensing. C) Some strains produce enzymes that destroy quorum sensing signals, thus inhibiting quorum sensing activation. D) Many variations of the quorum sensing signal acyl-homoserine lactone exist. Some versions activate quorum sensing and others inhibit quorum sensing. Data shown is the CviI/CviR quorum sensing system in Chromobacterium violaceum from [68]. BHL N-butanoyl-L-homoserine lactone, HHL N-hexanoyl-L-homoserine lactone, OHL N-octanoyl-L-homoserine lactone, OOHL N-(3-Oxo-octanoyl)-L-homoserine lactone, DHL N-decanoyl-L-homoserine lactone, ODHL N-(3-Oxo-decanoyl)-L-homoserine lactone, and dDHL N-dodecanoyl-L-homoserine lactone.

inhibits production of QscR, and QscR regulates LasI/LasR and RhlI/RhlR [62], [63]. Although many such regulatory interactions within the *P. aeruginosa* quorum sensing system have been elucidated [56], [63], our understanding of all such interactions and their roles in regulation is likely far from complete.

QscR, mentioned above, is a fifth quorum sensing receptor that also participates in quorum sensing regulation in *P. aeruginosa* [66]. Although the receptor is not co-expressed with a unique signal synthase, it is capable of binding 3O-C12-HSL made by LasI as well as other long chain HSLs that may be produced by neighboring species. QscR has a unique binding site on the genome, and is able to form complexes with both LasR and RhlR. It remains unclear how feedback between QscR and the four networks potentially broadens the capability of *P. aeruginosa* to dynamically respond to changes in cell density.

VII. Multispecies Signaling

Although the signaling molecules involved in these quorum sensing networks are specialized, there is potential for these signals to enable interspecies crosstalk. Crosstalk is the process in which a signal from one type of cell communicates with another type of cell, acting as either an agonist or antagonist of the receptor as shown in Fig. 7A and B. Several species produce signaling molecules that interact with receptors in another species [67], [68]. For example, the autoinducers produced by *P. aeruginosa* were able to activate quorum sensing within neighboring *Burkholderia cenocepacia*, although the autoinducers produced by *B. cenocepacia* did not activate the quorum sensing systems of *P. aeruginosa* [45].

C. violaceum has proven to be a useful strain to test the variety of responses elicited when different versions of homoserine lactone signals compete for the same receptor. Some strains of C. *violaceum* produce C6-HSL, which strongly activates the associated CviR receptor, but the presence of an additional type of HSL autoinducer modulated the ability of the CviR receptor to regulate gene expression [68]. As depicted in Fig. 7D short chain HSLs were able to activate CviR-regulated genes, whereas the addition of HSLs with a longer acyl chain inhibited the ability of the native C6-HSL to activate quorum sensing. Similar crosstalk has been observed between other HSL-based signals [69]. Each of these HSLs had different affinities for the CviR receptor and sometimes changed the activity of the receptors in subtle ways, such as changing the interaction between CviR and its binding site on the genome. Through signaling crosstalk, neighboring species, such as *B. cenocepacia* or *P. aeruginosa* are capable of interfering

with quorum sensing regulation in *C. violaceum* [70], [71]. Given the number of species producing different versions of HSL, it is likely that interspecies signaling interactions are more prevalent than is currently appreciated.

Other organisms are known to produce compounds that interfere with quorum sensing activation [72]. A classic example is the halogenated furanones produced by the alga *Delisea pulchra*. Early studies reported these compounds are capable of binding to and deactivating autoinducer receptors [73].

Another strategy to interfere with quorum sensing is to destroy or chemically modify the autoinducer before it reaches its receptor [72], as shown in Fig. 7C. AiiA isolated from *Bacillus* species is one such enzyme [74]. AiiA is a lactonase that cleaves the lactone ring of HSL autoinducers, rendering it unable to bind to its receptor. An oxidoreducatase from *Burkholderia* also alters the specificity of a signal for receptor molecules by removing a carbonyl group on the third carbon of the acyl chain of acyl-homoserine lactones [75].

VIII. Modeling Approaches

Several modeling approaches have been implemented to predict the impact of quorum sensing systems on gene expression dynamics. These models often examine different aspects of the length, timescales, and complexities of quorum sensing signaling, ranging from a full model of how an individual signaling system impacts the expression of a specific gene to how the diffusion of signals through space dictates expression patterns.

A. Coupled Differential Equations

A basic model for quorum sensing activation may simply be a set of coupled differential equations. These equations describe the production and degradation of synthase, receptors, autoinducers, and cells [76]–[79]. The activation of quorum sensing is typically modelled as a two-state system, in which the population switches on quorum sensing once the autoinducer level reaches a threshold concentration, although comparisons have been made between such all-or-none transitions and graded transitions [80]. For example, to follow the change in the number of receptors bound by autoinducer (B) over time as a function of number of receptors (R) and number of autoinducers (A), Dockery and Keener [76] proposed the following model:

$$\frac{dR}{dt} = V_R \frac{B}{K_R + B} + R_0 - k_R R, \tag{1}$$

$$\frac{dA}{dt} = V_A \frac{B}{K_L + B} + A_0 - k_A A, \tag{2}$$

and

$$B = \frac{k_{RA}}{k_b} RA, \tag{3}$$

where k_i's are rate constants, $\mathrm{V_i}$'s and $\mathrm{K_i}$ are Michaelis-Menten parameters, and $\mathrm{I_0}$'s are basal production rates. These equation demonstrate the typical setup of a quorum sensing model: non-linear, positive feedback of bound receptor on the levels of receptors and autoinducers and a basal production of both autoinducers and receptors, the dominant production term when signal concentration is low.

Some models incorporate multiple layers of quorum sensing regulation, such as a full model of the *V. harveyi* system [81]. Stochastic models have also been developed [77].

When moving beyond uniform and well-mixed systems, the spatial heterogeneity in cell position can be taken into account. A recent paper examined how the distribution of small colonies of *Pseudomonas syringae* on the surface of a leaf influenced quorum sensing activation [82]. Reaction diffusion models can be used to examine the exchange of autoinducers. In such models, the cells producing and responding to the quorum sensing signal are distributed in space. In addition to production and degradation of the signaling molecule, the signal spreads out by diffusion. Predictions using reaction-diffusion models of quorum sensing activation match well with experimental measurements in spatially distributed systems [83], [84]. Beyond diffusive transport, quorum sensing and autoinducer gradient formation have been examined in the presence of flow [85].

B. Simplified Models of Regulatory Networks

Systems biology has implemented a variety of approaches to examine how multiple, interconnected regulators work together. Some of the more abstract examples are Boolean networks to capture activation and repression of gene expression [86], [87]. A gene regulatory network is the sets of regulatory molecules, typically proteins but sometimes also regulatory RNAs, that control the production of proteins and RNA inside the cell. These factors often regulate one another, and this type of feedback is captured well with a Boolean network model. Boolean networks analyzed connections within complex regulatory networks, such as fly embryo development and yeast cell cycle [86], and should prove useful in dissecting interactions within multilayered quorum sensing systems.

In the most basic Boolean networks, regulatory components are either on or off. The bimodal states at the heart of Boolean networks are a close approximation to the nearly digital states observed for many regulatory decisions within a cell [88]. When incorporating signaling into these networks, the local signal concentrations enters in as a new state, in which the signal concentration is either above or below a threshold concentration for activation [87]. These models have been used to explore how multiple quorum sensing networks work together to regulate gene expression. Recent papers examined the quorum sensing networks of *P. aeruginosa* using such rule-based models [89], [90]. These models take into account full complexity of the interactions within these networks.

C. Ecosystem-Level Models

When considering interactions amongst multiple species, many of which might be poorly characterized at the level of molecular mechanisms, agent-based or individual-based models have been applied. In these models, each type of cell follows a set of rules that governs its interactions with other species. Over time, the behavior of each individual within the population is calculated following these rules. These rules generally simplify the interactions to a large extent, but have been able to reveal emergent properties of these systems, such as robustness and bistability [91]. Recent success in applying ecosystem level modelling revealed how pairwise

interactions between species in the gut microbiome influenced the progression of *Clostridium difficile* infections [92].

Although many multispecies interactions are known [93], we are still just beginning to explore signaling interactions within multispecies systems. Many natural microbial systems are highly diverse, competing and cooperating with hundreds to thousands of species, suggesting that many regulatory decisions mediated by extracellular signals will be influenced by neighboring cells. New software tools such as BSim should aid in understanding the collective behavior of multispecies bacterial populations [94], [95].

D. Quorum Sensing and Information Theory

Recent work has implemented the techniques of communication and information theory to analyze cellular signaling networks, including quorum sensing. Examining quorum sensing through the lens of communication and information theory has the potential to give insight into the design principles of cellular signaling networks, including limitations on information exchange rate, the role of and sources of noise in signal transmission, and integration of multiple signals at a receiving cell. Entropy and mutual information measures have been applied to experimental data [19], [96], [97] in the hopes of finding insights into behavior. While these works do explicitly discuss signaling dynamics, the information theoretical analysis does not consider a time-varying system. From a more communications perspective, the impact of signaling errors and the potential for QS to synchronize molecular communication systems have been investigated [98], [99]. The analysis of information theoretic capacities for systems where multiple molecular bindings occur and other multicellular processes relevant to QS have been presented in [100]. Tools to efficiently simulate signaling within complex cellular networks have been developed [95], [101]. The examination of QS from a control theoretic viewpoint (e.g., stability analysis) is more prevalent [102]–[104]. A different track using queues as models for the collection of key molecules in QS is provided in [105] motivated by the successful use of queues in modeling the behavior of electron transfer in bacteria [106], [107].

IX. Applications

A. Targeting QS as a Therapy

Understanding quorum sensing regulation at a more quantitative level has many potential applications in human health and bioengineering. The medical connection to quorum sensing stems from the fact that many virulence mechanisms used by bacteria to help pathogens invade the body are quorum sensing regulated [18], [108]. Quorum sensing enables pathogens to recognize when they are sufficient numbers to mount a successful attack or evade the body's defenses. For example, in animal model studies quorum sensing increases the ability of *P. aeruginosa* infections to spread throughout the body [108]. In another example, quorum sensing helped *Staphylococcus aureus* detect when it has been captured by a host cell and internalized into an endosome [109]. Confinement leads to the accumulation of the autoinducer and activates quorum sensing [110], [111]. Activation of quorum sensing allows the cell to escape the endosome and potentially replicate intracellularly [109], a process that contributes to persistence [112]. Quorum sensing activation is often a critical component of the pathogen successfully invading the host, suggesting that interference with signaling may be sufficient to prevent or treat many bacterial-related health problems [18].

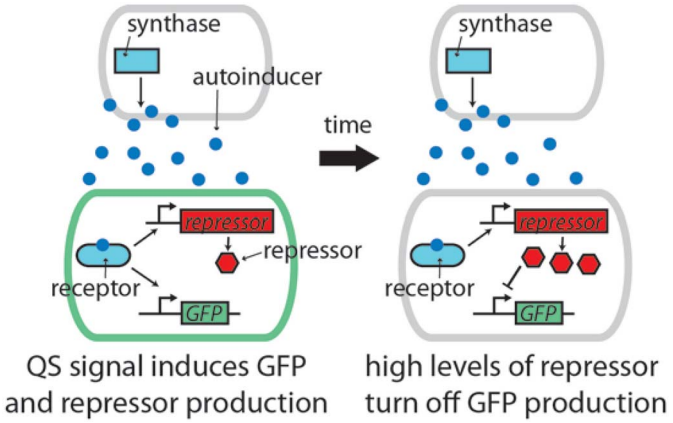

Fig. 8. Quorum sensing systems have been used in synthetic gene circuits to mediate communication between cells. In one such system autoinducer produced by synthase in a sender strain induces expression of GFP and repressor in a receiving strain. Continued production of repressor over time eventually shuts off GFP production in the cell, leading to a pulse of GFP production. Adapted from [122].

Blocking quorum sensing activation may help to prevent or treat microbe-associated diseases. Drugs that prevent virulence activation as opposed to killing microbes have gained attention in part due to concerns over the rise of antibiotic resistance. Antibiotics that kill microbes elicit a strong selection pressure for resistance. Resistance to treatment may be less likely to evolve when microbes are instead "disarmed" by reducing virulence [113], [114].

Specific inhibitors of many quorum sensing systems have been identified. These compounds have a variety of mechanisms, from interfering with receptor binding to deactivating the signals through cleavage or chemical reactivity [113]. Many of these compounds are naturally occurring, such as the furanones produced by the alga *Delisea pulchra* discussed above. High throughput screens of chemical libraries have also revealed synthetic compounds that inhibit quorum sensing [72], [115], [116].

B. Quorum Sensing to Control Synthetic Biological Systems

In recent years quorum sensing has impacted bioengineering and synthetic biology. The systems of *P. aeruginosa, V. fischeri,* and *V. harveyi* in particular have been particularly useful as a model signaling mechanism to incorporate into laboratory organisms such as *E. coli* and yeast [69], [117], [118]. Using quorum sensing systems as a building block of artificial genetic circuits enables cells to communicate over long distances and respond to changes in cell density. Circuits involving quorum sensing have enabled fine tuning of *E. coli* population sizes, using high density to activate toxic pathways in the cells that reduce populations levels back to sub-threshold concentrations [119]. In another application, the quorum sensing systems of *P. aeruginosa* were split between two different strains of *E. coli*, enabling bidirectional communication and

programmed pattern formation [120]. Quorum sensing has also been used as part of a circuit that enables microbes to detect the boundary between light and dark [121] and to generate pulses of gene expression, as depicted in Fig. 8 [122]. A better understanding of the capabilities of these networks, particularly regulation involving the integration of multiple signals, should enable design of genetic circuits with increased complexity. The incorporation of new quorum sensing systems into synthetic cells will potentially expand our ability to program cell-cell signaling [123].

X. Conclusion

Inevitably the list of quorum sensing systems will continue to grow, with pathways being discovered in known species and new species shown to create and respond to the accumulation of extracellular signals [42], [124]. The increased availability of whole genome sequences will also assist in the ability to identify and characterize novel quorum sensing systems and perhaps predict how they fit into the larger regulatory context of the cell. As the catalogue of parts grows, there is greater need to predictably understand how these networks of interconnected signals work. Parallel development in quantitative models of signal exchange, at all levels of complexity, will reveal new possibilities in manipulating these signaling networks in the wild as well as designing synthetic systems with more sophisticated responses.

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