Understanding Bacterial Cell−Cell Communication with Computational Modeling

Andrew B. Goryachev*

Centre for Systems Biology, School of Biological Sciences, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR, United Kingdom

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Andrew Goryachev is a computational cell biologist with a multifaceted background and broad interests in the dynamics of cellular regulatory networks. Trained as a biophysicist at the Moscow Institute of Physics and Technology, he received his Ph.D. in theoretical computational Chemistry at the University of Toronto. Presently, he is a Lecturer at the Centre for Systems Biology, University of Edinburgh, UK. He has a long-standing interest in bacterial quorum sensing to understanding of which he contributed by developing computational models on intracellular as well as population-wide scales.

1. Introduction

In the past decades, bacterial cell−cell communication has captivated interest of a broad scientific community drawn from a wide spectrum of disciplines including biology, physics, chemistry, mathematics, and engineering. Extensive exchange of experimental techniques and theoretical paradigms resulted in burgeoning development of the field as well as inevitable mixing of research cultures. As is often the case when multiple disciplines address a complex scientific problem, mathematical equations can provide a unifying platform which synergizes the efforts. Indeed, integration of many disparate experimental results in the form of models that span multiple scales from molecules to populations has already greatly benefited the field. In the present contribution, I will briefly survey the key developments in the rapidly growing field of modeling approaches toward understanding bacterial cell−cell communication on a systemic level.

Complex prokaryotic metabolism generates a diverse array of chemicals that enter the extracellular environment and can potentially function as signaling molecules. The list of bacteria-produced substances known to function as cell−cell communication signals grows constantly[1,2] and is likely to continue expansion in the foreseeable future. Once outside the bacterial cell, these molecules find themselves in diverse, often hostile, environments where they freely diffuse until adsorbed to surfaces, chemically degraded, assimilated by other organisms, or perceived by potential signal recipients. Even in the absence of degradation, the intensity of this undirected, diffusion-propagated chemical signal rapidly falls with the distance from the signal source. Thus, success of any cell−cell communication mediated by freely diffusing molecules, defined as the ratio of received to the total number of secreted molecules, strongly depends on the characteristic cell-to-cell distance. Apart from some exceptional situations in which bacteria might find themselves enclosed in tiny diffusion-impermeable compartments (see section 3), this implies that cell−cell communication becomes a significant factor only when the local cell density reaches certain threshold level. Not surprisingly, most of the known cell−cell signaling and communication in bacteria is cell density dependent. In the majority of examined systems (a few notable exceptions have also been characterized, see e.g., ref 3 and discussion in section 7), the mode of bacterial cell−cell communication is autocrine, i.e., cells capable of producing the signal are also the cells that can receive the signal. The received signal is directly translated into a change in transcription regulation, the decision-making level of a prokaryotic cell at which cell−cell communication is integrated with other sensory inputs. The ability of bacteria to regulate gene expression programs in response to autocrine diffusible signals is typically referred to as quorum sensing,

* To whom correspondence should be addressed. E-mail: Andrew.Goryachev@ed.ac.uk. Phone: 44-131-650-7807.

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which is by far the most explored mode of bacterial cell−cell communication and, thus, is the major focus of this review.

Since its first observation in marine luminescent bacterium *Vibrio fischeri* in the early 1970s,[4] quorum sensing (QS) has been attracting ever increasing attention.[2,5−12] With the increased availability of sequencing technologies, it became clear that homologues of key *V. fischeri* QS genes are present in the genomes of many Gram negative bacteria.[6] Independently, QS has been described in several Gram positive species, further highlighting the panprokaryotic nature of this phenomenon. Ubiquity, cooperative nature as well as the ecological and medical significance of QS ignited the interest of researchers in physical and mathematical disciplines. Being simple unicellular organisms, bacteria have a short path from extracellular signals to gene regulation and from switching gene expression programs within individual cells to changing behavior of the whole populations. This opens an unprecedented opportunity for the systems and synthetic biology studies in which technically feasible genetic alterations can be predicted in silico to produce specific behavioral changes, and these theoretical predictions can then be readily tested in vivo (see ref 13 for review and the discussion in section 6). As a result, realistic quantitative models can be built in a timely fashion through several converging iterations of experimental and theoretical work. Success of such integrated efforts has been greatly facilitated by the design of genetically encoded fluorescent reporters and advancement of fluorescent microscopy that nowadays permit measurement of gene expression within individual cells.[14] Being tractable experimentally, bacteria are also attractive targets for theoretical analyses. Thus, bacteria have relatively well-understood modes of gene regulation and short signal-transduction cascades frequently consisting of only one or two levels. Small sizes of bacterial cells often permit ignoring of spatial heterogeneity and thus reduce the complexity of modeling the intracellular environment to ordinary differential equations and space-independent stochastic methods. Beyond these features, QS has an additional appeal to theoreticians. Cell−cell communication by means of mathematically well-defined diffusion-mediated transport allows for creation of spatially explicit models of whole populations in diverse habitats. This has been achieved by using either continuous description, such as partial differential equations, or discrete agent-based methods. Combining mathematical description of intracellular molecular networks with that of population-wide cell−cell communication provides for insightful multiscale dynamical models capable of predicting outcomes of complex experiments in which both intracellular and ecological factors are varied.

The following content is subdivided as follows. In section 2, I focus on the structure−function relationship in the organization of quorum sensing gene networks whose molecular details have been studied in some detail. Section 3 changes the perspective from the mechanisms of implementation to the ecological and evolutionary function of quorum sensing. The role of quorum sensing within bacterial biofilms is the subject of section 4, while biomedical applications related to suppression of cell−cell communication in pathogenic bacteria are considered in section 5. A novel field of research, prokaryotic synthetic biology that uses controlled cell−cell communication to achieve desired patterns of population-wide behavior, is introduced in section 6. To complement the main theme of this review, in section 7, I discuss a few known examples of cell−cell communication that do not fall in the wide category of quorum sensing phenomena. Finally, some unsolved problems and potential future directions are highlighted in section 8.

2. Quorum Sensing Gene Networks: From Design to Function

Experimental and theoretical accessibility of QS made it a subject of choice for studies on environmentally regulated gene networks. One common theme that motivated much of the work in the field is how the architecture of the quorum sensing gene network (QSN) translates into its function as a sensor and an element of bacterial decision-making machinery. A host of associated questions has been raised and vigorously discussed in the literature. Is QSN a true switch with distinct "on" and "off" states? Which elements of the QSN are responsible for the switch-like behavior? What defines the critical level of extracellular signal that turns QSN on? How does this critical level of signal translate into the bacterial density and, indeed, how many cells constitute a quorum? How do QSNs operate in the conditions of extreme molecular noise that stems from the microscopic size of a bacterial cell? These and other questions focusing on biochemical and biophysical properties of QS necessitated the use of quantitative modeling and, in fact, have been found particularly amenable to the combined experimental-theoretical analyses.

2.1. Are Quorum Sensing Networks "Switches" or "Rheostats"?

All components of so far characterized QSNs can be attributed to three broadly defined functional groups: (1) signaling molecules, termed autoinducers (AIs), their synthases and machinery for AI processing and secretion; (2) optional AI receptors, signal transduction elements and intermediate transcription regulators; (3) QS transcription regulators (QSTRs) - master transcription factors that control expression of target genes. As the main goal of the QSN is to regulate the copy number of QSTR(s) in response to the extracellular concentration of the AI(s), its physiological activity can be insightfully characterized by the shape of the QSTR(AI) dose−response curve. The network may operate as a rheostat (see Figure 1A,B) by gradually increasing the copy number of the transcription factor. This behavior is typically characterized by an S-shaped curve exhibiting a range of AI concentrations within which the QSN responds to the stimulation with AI. Curves of this type are commonly fitted to the Hill function of the form

$$\frac{A \times x^n}{B + x^n}$$

If the Hill coefficient n is greater than 1, the behavior is often said to be "ultrasensitive"[15] as shown in Figure 1B. Importantly, in this regime, the network has only one possible output for each input value and the S-shaped curve has a positive slope everywhere. Alternatively, the QSN may exhibit bistability with two disjoined branches of stable steady states, often referred to as "on" and "off", which are separated by experimentally undetectable unstable state.[16] In this scenario shown in Figure 1C, QSN operates as a true genetic switch and the cells are expected to be found in either the uninduced "off" state or the fully induced "on" state.

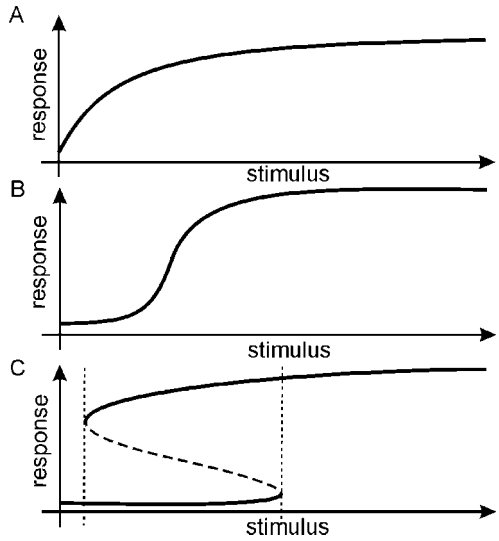

Figure 1. Characteristic shapes of dose−response curves observed in gene regulatory networks. (A) Gradual response with Hill coefficient $n = 1$ corresponds to the standard Michaelis−Menten curve. (B) Ultrasensitive rheostat-like response, $n > 2$, is characterized by a typical sigmoid curve that remains a single-valued function of the stimulus even at very large n. (C) Bistable behavior is represented by two branches of steady states separated by an unstable state branch (dashed line) within the stimulus interval shown by two dotted vertical lines.

This sets it apart from the first case in which the majority of the population is likely to exhibit intermediate expression levels.

Gram negative QSNs discovered early in the history of the QS research, such as the paradigmatic *luxR/I* network characterized first in *V. fischeri*, seemed to present a particularly simple design. Indeed, QSTRs of the LuxR type, as many other bacterial transcription factors, are also simultaneously receptors for their cognate signaling molecules, *N*-acylhomoserine lactones (AHLs).[17] AHLs are synthesized from commodity cellular metabolites by several enzyme classes, of which LuxI is the best characterized.[18,19] Despite intracellular localization of their receptors, cell−cell communication with AHLs is possible because most AHLs can freely diffuse in and out of the cell through both membranes and the cell wall. However, additional QSN elements, such as efflux pumps, might be required if AHL's passive diffusion transport is hindered by their long acyl chains (>10).[20]

The *luxR/I* type of QSN, the layout to be first thoroughly analyzed experimentally, became also the testbed for the development of modeling approaches directed at quantitative understanding of QS (see Figure 2 and box 1 in ref 21 for details and definitions). The network's fundamental property, a positive feedback loop by means of which AI enables transcriptional activity of the QSTR that, in turn, positively regulates the AI synthase (Figure 2A), has been proposed as the first mechanistic explanation of the QS phenomenon[5] and, not surprisingly, became the focus of the early modeling efforts. In mathematical disciplines, existence of positive feedback has long been known to be a necessary condition for bistability.[16,22] Two studies, by James et al. on *luxR/I* QSN in *V. fischeri*[23] and by Dockery and Keener on *lasR/I* network in *Pseudomonas aeruginosa*,[24] for the first time applied the language of ordinary differential equations to formulate mechanistic assumptions underlying the purported functionality of the *luxR/I*-type network. Both studies

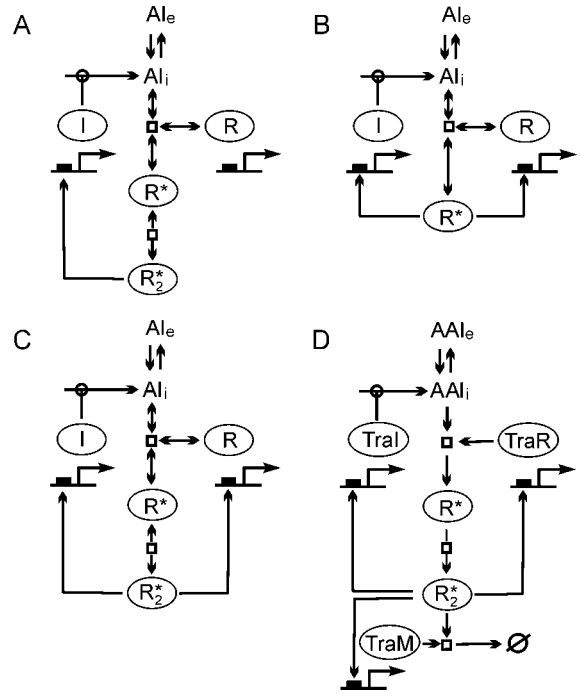

Figure 2. Layout of the classical *luxR/I* QSN with known variations. (A) A commonly reported layout with QSTR dimerization and single positive feedback loop. (B) Layout without dimerization but with two positive feedback loops was the first to be considered theoretically.[23,24] (C) Presence of QSTR dimerization and second positive feedback loops together was shown to provide increased robustness of QSN switch-like behavior to noise.[25] (D) The irreversibility of the QSTR formation was proposed to necessitate an additional negative feedback loop based on the antiactivator TraM in *A. tumefaciens* QSN.[37] Protein species are shown as ovals; R, luxR-type QSTR; I, LuxI-type AI synthase; AAI, Agrobacterium AI. Reactions of complex formation are shown by open boxes, ∅ symbolizes degradation, and catalytic action of LuxI enzymes is depicted by open circle-ended arrows.

concluded that, in principle (due to the absence of experimentally measured network parameters the analyses had to remain qualitative), the network layout permits bistability with distinct "on" and "off" states. These results suggested a tantalizing possibility that the *luxR/I*-type QSN comprises a true genetic switch and inspired a number of efforts attempting to predict bistability in other QSN layouts in silico and find experimental evidence for or against this phenomenon in vivo.

It is, however, important to note that the existence of the "classical" positive QS feedback loop from LuxR to LuxI does not by itself necessitate the emergence of bistability. On the contrary, using simple kinetic reasoning,[21,25] it can be readily shown that the "bare-bone" network with a single positive feedback loop and monomeric transcription factor *cannot* be bistable with *any* combination of network parameters. Thus, network elements additional to the classical positive feedback loop are required to enable bistable behavior of the *luxR/I*-type network. Goryachev et al.[25] systematically addressed this question and concluded that either dimerization of the QSTR or an additional positive feedback loops are required to achieve bistability (cf. Figure 2A−C). Interestingly, both original studies[23,24] that suggested bistability of the *luxR/I*-type QSN in two different microorganisms explored the same scenario in which a monomeric QSTR positively regulates the AI synthase and itself as shown in Figure 2B. Thus, hypothesized positive autoregu-

lation of the QSTR provided second, additional positive feedback loop that brought about bistability.

Providing some support to the bistability hypothesis, ample evidence exists that many LuxR-type QSTRs operate as dimers. Moreover, activator-type LuxR homologues have been shown to become dimerization-competent only upon binding their cognate AHLs.[26,27] Dimerization, in turn, enables these QSTRs to bind upstream *cis*-regulatory elements of the target genes and activate their transcription. Even *P. aeruginosa* LasR, assumed monomeric in early modeling studies,[24,28] more recently was shown to be dimeric in vitro[29] and is likely active as a dimer or higher-order oligomer in vivo.[30] Evidence for the existence of additional positive feedback loops has also been presented in some systems. Thus positive autoregulation has been reported for QSTRs *carR* in *Erwinia caratovora*,[31] *traR* in *Agrobacterium tumefaciens*,[32] and *lasR* in *P. aeruginosa*.[33,34] Originally described in ref 35, positive autoregulation of *V. fischeri luxR* has been revisited recently by Williams et al.[36] In an elegant study, they combined theory and experiment to provide additional evidence in support of *luxR* positive autoregulation and the existence of bistability in the classical *luxR/I* network.

Fundamental limitation for the *luxR/I*-type network layouts is the requirement for the relative instability of the QSTR dimer and reversibility of the QSTR-AI interaction.[37] Indeed, due to the constitutive intracellular production of AI and translation of the QSTR, strong interaction between the monomeric QSTR and AI followed by largely irreversible QSTR dimerization could result in undesirable network "short-circuit"—accumulation of active transcription factor and eventual transition to the induced state even in the absence of cell−cell communication. One possible evolutionary solution avoiding this "runaway" activation is to keep the interactions between the QSTR and AI and between the two AI-bound QSTR monomers weak. This assumption, implicitly made in the majority of *luxR/I*-type network models, has been experimentally confirmed in some species[38] but clearly disproven in others. Perhaps the best characterized counterexample is provided by the quorum sensing system of Ti plasmids in *A. tumefaciens*[32] in which QSTR TraR forms essentially irreversible complex with its cognate AI during translation.[26] Moreover, if translated in the absence of AI, TraR is poorly folded and insoluble. Experiments in *A. tumefaciens* identified a protein, TraM, whose deletion resulted in constitutively induced QSN state.[39] Paradoxically, TraM was found to be positively regulated by the very TraR.[40] Modeling analysis of the *traR/I* network by Goryachev et al.[37] resolved this seeming contradiction and demonstrated that TraR−TraM negative feedback loop shown in Figure 2D is indeed the network element that enables sensing extracellular AI despite the irreversible interaction of QSTR TraR with its AI. This study also predicted bistable, switch-like mode of operation for the *traR/I* QSN. Interestingly, *P. aeruginosa* LasR was found to bind its cognate AI 3OC12-HSL with picomolar-range dissociation constant while LasR, translated in the absence of AI, was largely insoluble.[29] These features, reminiscent of TraR and its interaction with the cognate AI, suggest that mechanisms similar to those characterized in *A. tumefaciens* might be at play in the highly complex QSN of *P. aeruginosa*.

Although *V. fischeri*-type *luxR/I* network remains probably the most popular example of QSN, many more network layouts have been discovered and characterized to various

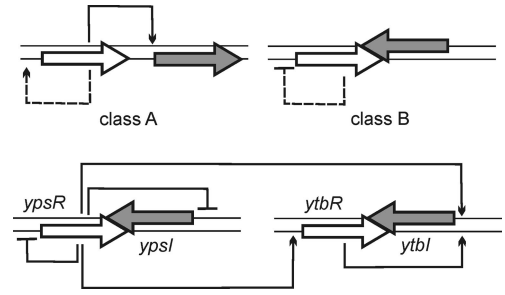

Figure 3. *luxR/I* QSNs of Gram negative bacteria belong to two distinct classes with different genomic layout and mode of regulation (see text). A complex regulatory pattern described in *Yersinia pseudotuberculosis* has been attributed to the interaction between its two sequentially connected class B QSNs.[43] Arrowheads represent transcriptional activation, and hammerheads represent repression. Elements reported only in some organisms are shown by dashed line. Open arrows indicate QSTR genes, and gray arrows depict AI synthase genes.

degrees of detail (see refs 21 and 41 for recent reviews on the QSN architecture and design principles). Analyses of *luxR/I* homologues in some γ-proteobacteria demonstrated that despite sequence similarity with *V. fischeri* genes, their functional mode of operation is fundamentally different, warranting separation of these QSNs into a distinct functional class[21,42] that is also characterized by different genomic organization as shown in Figure 3. QSTRs of this network type (class B) are generally dimeric repressors that negatively regulate transcription of their target genes in the absence of their cognate AIs. Binding to the AIs induces conformational changes that reduce affinity of the QSTRs to DNA and relieves repression of the target genes in the state of quorum. These QSTRs frequently repress themselves, but in the most reported cases, they do not regulate the respective AI synthases.[42] Therefore, mechanisms based on the "classical" positive QS feedback loop and positive autoregulation of QSTRs characterized in *V. fischeri*-type (class A) networks are not applicable to this class of QSNs. Although complex regulatory patterns have been suggested by some studies[43] (see Figure 3), more experimental and theoretical work is required before their mode of operation can be fully understood.

Of other functionally distinct QSN layouts in Gram negative bacteria, perhaps the best characterized are networks of *Vibrio* species. QSTRs LuxR in *V. harveyi* (not related to *V. fischeri* LuxR) and HapR in *V. cholera* belong to a large family of TetR transcription factors. They do not bind AIs but instead are regulated by sRNA species downstream of the cell-surface AI receptors.[44,45] Intricate architecture and functional principles governing *Vibrio* QSNs have been unraveled recently in a series of insightful papers by Bassler and colleagues (see refs 46−49 and the discussion in section 2.3).

Cell−cell communication in Gram positive bacteria mostly relies on genetically encoded autoinducer peptides (AIPs).[50,51] Translated as precursors, these peptides are proteolytically processed and extruded into the extracellular space by enzymes positioned in the cell membrane. Reception is achieved by either transmembrane receptor histidine-kinases or through transporter-facilitated import into the cell where peptides are recognized by intracellular receptors, such as *Bacillus subtilis* phosphatase Rap.[52] Prototypical example of a peptide-based Gram positive QSN is provided by the *agr* system in *Staphylococcus aureus*,[53] which is schematically shown in Figure 4A. The *agr* operon encodes the messenger

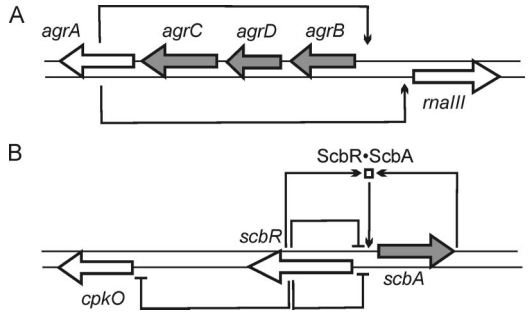

Figure 4. Known and hypothesized QSN layouts of Gram positive bacteria that were predicted to exhibit bistability. (A) *agr* operon of *S. aureus* is autoregulated by the QSTR AgrA that also activates transcription of the regulatory RNA species *rnaIII*. (B) A complex regulatory layout that has been suggested to function in *S. coelicor*.[66] Open arrows indicate QSTR genes, and gray arrows depict other regulatory genes.

molecule AgrD (precursor), processing enzyme AgrB, transmembrane histidine kinase AgrC, and the response regulator AgrA. Once phosphorylated by receptor AgrC, AgrA activates transcription of the *agr* operon and that of the regulatory RNA species, RNAIII, which serves as the ultimate effector of the QSN instead of AgrA. The ingenuous, functionally economical design of the *agr* QSN with three overlapping positive feedback loops (AgrA positively regulates AIP, AIP receptor and itself) suggests complex nonlinear behavior of the network with a potential for bistability. Indeed, Gustafsson et al.[54] modeled the dynamics of the *agr* network and theoretically predicted its bistable character. A later work by the same group addressed regulation of genetic competence (ability to incorporate exogenous DNA) by the *com* QSN in *Streptococcus pneumoniae*.[55] In addition to predicting bistability of the *com* QSN, they also suggested that a negative feedback loop may be responsible for the experimentally observed oscillatory onset of competence. The dynamics of the *S. aureus agr* operon was recently revisited from a more mathematical viewpoint by Jabbari et al.[56]

Increasing number of studies in prokaryotes characterize QS systems that rely on molecules other than AHLs or peptides to convey messages between the cells. These include quinolones,[57] fatty acid methyl esters,[58] and DSF (*cis*-11-methyl-2-dodecanoic acid)[59] in Gram negative species and γ-butyrolactones[60] in Gram positive. In addition, both types utilize a family of furanones, collectively known as AI-2,[61] for interspecies, pan-prokaryotic communication.[62] Modeling analyses of AI-2 network in *E. coli* have revealed significant complexity of AI-2 production and uptake[63−65] and suggested that AI-2 may play multiple roles in bacterial physiology. While experimental characterization of the majority of these systems is presently incomplete, in some cases it is nevertheless sufficient to initiate mathematical modeling that generates insightful experimentally testable predictions. Mehra et al.[66] have recently analyzed γ-butyrolactone-regulated QSN (see Figure 4B) that controls antibiotic production in *Streptomyces coelicor*. Similar to the *agr* operon in *S. aureus*, the "classical" functional role of the QSTR is split between two entities (proteins in this case), CpkO, that activates transcription of target *cpk* gene cluster, and ScbR that participates in the AI-mediated QS regulatory loops. In a layout analogous to that of class B *luxR/I* QSNs, transcriptional regulator ScbR controls expression of the target genes by repressing *cpkO*, the amplifier *scbA* and itself. Presumed functional role of ScbA is, however, unique, as it is hypothesized to be both an enzyme involved in the synthesis of γ-butyrolactone SCB1 and also a regulatory protein that activates its own transcription by forming a complex with ScbR. At high concentrations, SCB1 binds to the *C*-terminus of ScbR and induces its dissociation from DNA. This leads to derepression of *scbA* and increased production of ScbA and SCB1. Presumably, as more copies of ScbA become available, more ScbR molecules form activatory ScbR·ScbA complexes. Interestingly, because ScbR is an autorepressor, transcription of *scbR* is derepressed concomitantly. However, in the presence of SCB1, the repressor role of ScbR is switched to that of activator by forming the complex with ScbA. Mehra et al. predicted that this ingenuous QSN design functions as a bistable switch, however, more experimental work is required to substantiate the mechanistic assumptions of their model and test its predictions. A recent study by Brown bravely ventured into the almost unchartered waters of the Gac/Rsm networks.[67] This regulatory motif that consists of the two-component GacS/GacA element and the protein−RNA RsmA/RsmB module has been characterized in several microorganisms and is known to be involved in QS in the context of the class B *expR/I* QSN in *E. caratovora*.[21,68] Using detailed deterministic and stochastic modeling, Brown, however, demonstrated that this motif on its own can explain QS behavior in *Pseudomonas fluorescens* and predicted bistability in this newly characterized QSN layout.

Relative to the abundance of in silico predictions of bistability in a variety of QSN layouts, there is paucity of experimental studies testing the existence of bistability in vivo. In part, this can be attributed to the technical challenges of performing expression essays with single-cell resolution. Modeling of the transition to quorum on a population scale using stochastic agent-based simulation environments[37,69] suggested that individual cells undergo the transition at widely varying concentrations of extracellular AI. A recent study in *V. harveyi* used fluorescent microscopy with single-cell resolution and was indeed able to detect this population-wide behavioral heterogeneity.[70] Thus transition to quorum by the entire population is spread over a range of cell densities, and any experimentally measured population-averaged value will inevitably report gradual, rheostatic transition even in the presence of intracellular bistability. To circumvent population averaging, a fluorescent reporter is inserted genetically downstream of the promoter of interest and the activity of the reporter is then recorded at various cell densities and concentrations of the exogenously added AI on a cell-by-cell basis. Bimodal distribution of cellular fluorescence intensity, corresponding to the distinct subpopulations of cells in the "off" and "on" states, is taken as a proof of bistability[71] (however, see ref 72). Using this approach, Waters and Bassler[48] investigated response of *V. harveyi* QSN to various AIs and their combinations and found no evidence for bistability. Instead, they found a clearly unimodal distribution indicative of the rheostatic mode of QSN operation. In support of this conclusion, Bassler and colleagues recently provided additional evidence by measuring single-cell dose−response curves of LuxR copy number that exhibited typical rheostat-like profile.[73] Moreover, the value of Hill coefficient, $n \approx 1$, found in these experiments also precludes the possibility of ultrasensitivity which was earlier theoretically predicted by the same group.[46] While these results demonstrate that bistability is not required for the functionality of all known QSNs, they also do not exclude the possibility of bistability in other network layouts.

Indeed, although integrative model has not yet been constructed for *V. harveyi*-like QSN, interactions within this network discovered and characterized so far do not suggest bistability also from the theoretical point of view. Analyses with single-cell resolution, similar to that in ref 48, are desirable in the systems where theoretical prediction of bistability had been provided. To date, the study by Levchenko, Stevens, and co-workers[36] remains the sole positive experimental verification of bistability in a bacterial quorum-sensing network.

2.2. Quorum Sensing in the Presence of Molecular Noise

A typical *Escherichia coli* cell with length 2 μm and diameter 0.5 μm has a cell volume of only $\sim$0.72 μm^3 (or 7.2×10^{-16} L). Therefore, for a signaling molecule freely diffusing in and out of the cell to be present within the cell at only a single copy, its concentration in the extracellular environment should be $\geq$2.3 *nM*. So-called slim rods, e.g., *A. tumefaciens*, and Gram positive cocci are even smaller ($V \approx 0.18\ \mu m^3$). For them, the minimal detectable concentration of an extracellular molecule is on the order of 10 nM. Accordingly, reception of a freely diffusing cell−cell communication signal is subjected to intense extrinsic molecular noise which arises solely due to the small size of the "detector", the bacterial cell itself, and not because of fluctuations in the extracellular concentration per se. Another important and well-known factor that affects bacterial gene regulation is the intrinsic molecular noise, which in part arises due to fluctuation of the copy numbers of the key participating molecules, in particular transcription factors.[74] Experimental observation of these fluctuations became possible chiefly due to the introduction of genetically encoded fluorescent reporters and the associated detection methods (see discussion in section 2.1). These technological developments enabled the giant leap forward from the inevitably population-averaged picture provided by the classical biochemical methods to the full appreciation of highly stochastic nature of bacterial intracellular environment.

In the framework of QS, both the extrinsic noise (detected AI) and the intrinsic noise are highest in the low population density state. With only a handful of potentially detectable AI molecules and their intracellular receptors per cell (e.g., in the case of *luxR*/*I*-type networks), stochastic fluctuations are greater than the average values that are normally interpreted as deterministic "concentrations". This makes a bacterial cell, particularly in the low population density state, a fundamentally stochastic system. As the dynamics of such systems generally has been shown to depart from that predicted by purely deterministic methods,[75,76] stochastic modeling techniques are required to confirm, refine, or refute the predictions of deterministic models. A number of studies[25,37,77] used the stochastic simulation algorithm (SSA), also known as the Gillespie algorithm,[78] to simulate intracellular dynamics of QSNs numerically. To account for molecular noise, Li et al.[63] adopted stochastic Petri nets (SPN) for modeling AI-2 network in *E. coli*. Interestingly, taking noise into consideration demonstrated that some predictions from purely deterministic models may need a revision. Goryachev et al. used SSA to study a number of *luxR*/*I*-type network layouts with increasing complexity.[25] They found that, with biologically realistic network parameters, some of the tested layouts, while predicted bistable by respective ODE-based models, failed to exhibit bistability in the presence of molecular noise which essentially obliterated the difference between the "on" and "off" states. They concluded that seemingly redundant network elements, e.g., multiple positive feedback loops, often found in the real-life QSNs, might be there to provide sufficient quantitative difference between the distinct states of the network to enable its robust, noise-resistant operation. Not only can noise negate some predicted QSN properties, it may potentially also bring about novel behavior, unaccounted for deterministically. Using a synthetic biology strategy, To and Maheshri have recently demonstrated bimodal population distribution of a transcription factor copy number in a yeast-based system where deterministic model did not warrant bistability.[72] In a theory−experiment systems study in *E. coli*, Tozaki et al. tested the applicability of ergodicity principle, an assumption that the percentage of cells in a given state is identical to the probability to find a cell in this state.[79] They found that ergodicity, typically taken for granted, may be readily broken if the bacterial growth rate is dependent on the network state, which is a reasonable assumption in the case of QS. Thus to avoid erroneous prediction and interpretation of experiments, stochastic effects arising from the molecular noise and random switching of QSN states require careful consideration.

To extend stochastic methodology to whole populations, Goryachev and colleagues[37] designed a parallel agent-based simulation environment in which individual cell-agents, whose intracellular QSN dynamics was simulated using SSA, were able to randomly move, divide, and exchange signaling molecules with the environment. Cell−cell communication was enabled by the common extracellular space in which diffusion of signaling molecules was treated in a continuous deterministic approximation. This unique tool permitted stochastic simulation of the transition of a whole population of *A. tumefaciens* to the induced, high population density state. Results of these simulations revealed astonishing diversity of individual cell behavior even in a spatially homogeneous extracellular environment, the phenomenon which was recently observed also experimentally.[70] As the computing power becomes more readily available, such computationally intensive methods may become viable and valuable tools for the analysis of cell−cell communication in complex habitats such as biofilms.

Are there any mechanisms embedded into the design of QSNs that allow them to harness molecular noise or at least reduce its harmful influence? Levchenko and colleagues noted that positive autoregulation of a *luxR*-type QSTR may play an additional noise reduction role because the intensity of the molecular noise in AI−LuxR interaction scales inversely with the square root of the copy number of LuxR in the cell.[36] Tanouchi et al.[80] highlighted a different noise reduction feature of QSNs, fast degradation of the QSTR in the absence of AI, which has been reported for a number of *luxR*-type QSTRs, such as *A. tumefaciens* TraR.[81] Zhou et al. theoretically analyzed a hypothetical cell−cell communication network inspired by QS but radically different from the natural QSNs by the inhibitory action of the QSTR−AI complex on the transcription.[82] This design exhibited spontaneous oscillations which could be synchronized between individual communicating cells in the presence of extracellular noise. Curiously, these synchronized oscillations were found to be the direct consequence of the extracellular noise and provided a potentially interesting example of a fundamentally noise-induced behavior whose biological relevance is yet to be demonstrated.

2.3. Integration of QSNs within Bacterial Decision-Making Circuitry

Cell−cell communication is an integral part of continuous environmental surveillance. However, molecular mechanisms responsible for the integration of cell-density dependent signals with other sensory information are only beginning to emerge from various studies in individual organisms (see ref 21 for review). Notably, the degree of QSN interconnectivity with other bacterial decision-making circuits is found to vary widely from case to case. Thus, *traR/I* QSN of *A. tumefaciens* controls replication and conjugative propagation of Ti plasmids and the absolute majority of the network components as well as the target operons are located on the plasmid itself.[32] This greatly decreases network interconnectivity and, as a result, the overall complexity of the system. On the other end of the complexity range lies diffuse, extremely interconnected QS system of *P. aeruginosa*, emphatically named by Greenberg and Schuster the "network of networks"[83] (see Figure 5A). Several studies cumulatively identified hundreds of genes involved in diverse bacterial life functions, such as basic metabolism, cell wall generation, stress adaptation, and DNA replication, as targets of this QS system.

Frequently, in the context of cell density-dependent communication, bacteria utilize multiple signaling molecules which are recognized by their respective receptors. The QS systems of such species then can be considered as supernetworks of several QSNs connected sequentially or in parallel. *P. aeruginosa* QS supernetwork is one of the best characterized examples of such highly interconnected QSNs. Its *lasR/I* subnetwork was shown to positively regulate *rhlR/I* QSN in a sequential arrangement.[33,84] Fagerlind et al.[28] modeled this hierarchical network layout and also considered some degree of its integration into a larger supernetwork by explicitly introducing cAMP-activated positive regulator Vfr. In addition to HSL signals synthesized by LasI and RhlI, *P. aeruginosa* also produces third cell−cell signaling molecule, quinolone PQS.[85,86] Transcription regulator PqsR (formerly MvfR) that positively regulates expression of the PQS-synthetic *pqs* operon is itself positively regulated by LasR. Thus PQS signaling system is integrated into the global QS supernetwork. Qualitative theoretical analysis of a PQS production-encompassing version of the ever-expending supernetwork was performed by Viretta and Fussenegger,[87] who sought to identify responses of the QSN to pharmacological interventions.

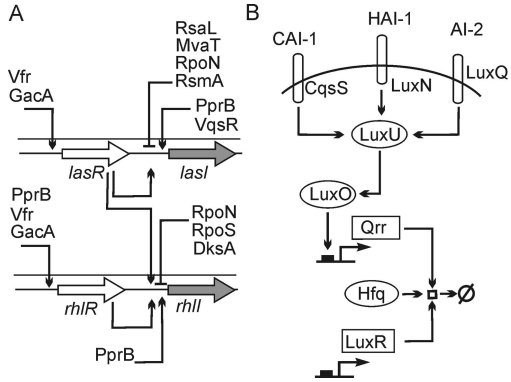

Figure 5. Integration of QSNs. (A) The two *luxR/I*-type QSNs of *P. aeruginosa*, *lasR/I*, and *rhlR/I* are sequentially connected and receive numerous inputs from various parts of cellular decision-making machinery (adapted from ref 160). (B) Three QSNs of *V. harveyi* are connected in parallel by feeding their input through the common LuxU−LuxO phosphorelay system that activates transcription of several species of Qrr regulatory RNA. In the presence of chaperone Hfq, Qrr create unproductive complexes with LuxR mRNA and, thus, block its activity. Protein species are shown as ovals and RNA species as rectangles.

QSNs organized in parallel are frequently found in *Vibrio* species[44] (see Figure 5B). Thus, *V. harveyi* uses three signaling molecules CAI-1, HSL HAI-1, and AI-2, which are recognized by their cognate cell-surface receptors CqsS, LuxN, and LuxP-LuxQ, respectively. Intriguingly, all three receptors utilize the same phosphotransfer protein LuxU and transcription regulator LuxO that controls expression of *qrr* regulatory sRNAs. Given this network layout, it is apparent that bacteria can sense only a weighted sum of the three individual input signals and cannot distinguish between separate channels. Using both experimental and theoretical methods, Bassler, Wingreen, and colleagues have studied this surprising evolutionary solution in great detail[73,88−90] and found that HAI-1 and AI-2 are combined strictly additively with nearly equal weights.[14] While contribution of CAI-1 remains to be determined experimentally, theoretical analysis of Mehta et al.[88] predicts that CAI-1 integration weight should be approximately equal to those of the other two inputs. On the basis of their information−theoretic approach, they motivated this prediction by the necessity to reduce signal interference between the three input channels.

A remarkable example of QSN integration with nutrient-sensing has been reported recently by Bischofs et al. in *B. subtilis*.[91] In this popular Gram positive organism, QS is mediated by Phr pentapeptides that upon import into the cell bind and inhibit activity of Rap phosphatases.[52] Although high cell density causes transition to sporulation, it is not sufficient to induce sporulation alone without concomitant starvation. The Bischofs et al. model suggested that as a result of integration between the QSN and the nutrient-sensing network, bacteria sense the amount of "food per cell" rather then absolute abundance of nutrition. Presumably, this clever design forewarns bacteria of the impeding starvation even when the local nutrition concentration is still satisfactory.

3. Why Do Bacteria Communicate?

The majority of the discussed so far aspects of quorum sensing and cell−cell communication in general were addressing questions of *how* diverse molecular mechanisms identified in various bacterial species enable them to sense and respond to the changing population density. Natural curiosity, however, prompts us also to ask questions such as *why* bacteria use quorum sensing. This question is directly related to the ecological function(s) and the evolutionary value of QS on the individual and population-wide scales. Since the early days of the QS research, it has been suggested that in the course of evolution, QS was selected for to control specific biological functions that are efficient only if performed by a large group rather then by separate individuals. Examples of such group-dependent actions include expression of virulence factors toward the common host, production of antibiotics, and generation of light. However, the need for collective action is not always obvious in specific cases of QS signaling. One of the best characterized QS systems controls conjugal transfer of Ti plasmids in *A. tumefaciens*.[32] Plasmid transfer by conjugation is a "private business" of the two involved cells, the donor containing the plasmid and the recipient who can harbor the plasmid but does not possess it yet. Presence of other donors does not increase the success

rate of any chosen donor cell per se. On the contrary, ability to sense the local density of potential recipients could have come handy by reducing the cost of unsuccessful transfers. This, however, is not possible because the QSN is located on the plasmid itself and thus all potential recipients are "QS silent"—they neither produce nor respond to the signal. Goryachev et al. addressed this paradoxical situation by explicitly simulating transition to quorum in distinct environmental conditions.[37] Specifically, they contrasted cell densities required to reach quorum in the batch culture where cells are distributed uniformly in suspension and in a more ecologically realistic assumption of growth as a biofilm. They found that while transition to quorum in the batch culture requires unrealistically high cell densities, transition in the biofilm can take place at the cell densities readily found in experiment. The reasons for this difference were clearly the more compact spatial distribution of cells and the loss of AI only into a half of the space in the case of biofilm. In conclusion, it was suggested that while Ti plasmids cannot "learn" about the density of potential recipients by using QS, they nevertheless can detect certain advanced stage of biofilm maturation. From that they can "determine" that their host cells are firmly attached within a dense bacterial mass which will inevitably also contain some potential recipients.

Analyses of cases, such as the above example, prompted the opinion that the concept of population "density-sensing" should be entirely replaced by "diffusion-sensing".[92] A specific example of a situation in which "density-sensing" is clearly irrelevant is the situation when a small number of bacterial cells is enclosed in a diffusion-impermeable compartment. *S. aureus*, a primarily extracellular pathogen, has been occasionally found within endosomes of nonprofessional phagocytes in the quantity of 1−2 cells per endosome. Internalized bacteria apparently manage to escape from the endosomes, proliferate in the cytoplasm, and eventually cause apoptosis of the infected cell.[93] Intriguingly, QS *agr* operon of *S. aureus* (see section 2.1 for details) is activated prior to the endosomal escape while the *agr*-defective mutant was found unable to proliferate inside the cells.[94] Koerber et al. modeled this peculiar instance of QS and computed analytically and numerically the bacterial escape time.[95] "Solitary confinement" of *S. aureus* cells was recently revisited experimentally by Carnes et al.,[96] who confirmed induction of *agr* operon during confinement of bacterial cells in the engineered nanostructured matrix and demonstrated enhanced survival of QS-competent cells. To unify "density-sensing" and "diffusion-sensing", a concept of "efficiency-sensing" was proposed recently.[97] This hypothesis, that essentially reiterated the conclusions of ref 37, postulated that cells compute overall efficiency of secreting extracellular molecules by factoring in cell density, spatial distribution of cells, and diffusivity of the medium. Although more biophysically balanced, this hypothesis also does not cover all possible functions of QS because secretion of diffusible extracellular effectors is not the universal "goal" of all known QS-regulated gene expression systems (conjugation of Ti plasmids is just one counterexample). Regardless of potentially subjective interpretations, QS allows bacteria to survey their environment by releasing and receiving diffusible signaling molecules.[98] Accumulating knowledge from numerous QS systems demonstrates that different species use this surveillance information in different ways, sometimes making entirely opposite decisions. Thus while some bacteria, e.g., *Pantoea stewartii,* build biofilm at high cell density[11] (collective action), others, like *V. cholera*, instead disassociate from the biofilm and become motile when "overcrowded". Particular decision will optimally suit specific needs and ecological strategy of a given microorganism. Given the diversity of ecological niches, the exact function of QS needs to be determined on a species-specific basis.

4. Quorum Sensing in Bacterial Biofilms

Biofilms, compact bacterial structures cemented together by extracellular matrix that form naturally on various surfaces and interfaces, have recently gained broad recognition as the major native prokaryotic habitat.[99−103] *Pseudomonas aeruginosa* is notoriously hard to eradicate with standard treatment protocols when it grows as a biofilm in medical catheters and in the lungs of cystic fibrosis patients. In 1998, Greenberg and colleagues suggested that QS plays a key role in the morphogenesis of *P. aeruginosa* biofilms.[104] They found that a mutant with genetically disrupted QS was showing abnormal biofilm morphology with concomitant increase in susceptibility to antimicrobials. This short report in *Science* magazine sparked a great amount of interest toward the role of QS in biofilm formation across several disciplines. Therefore, already the first theoretical studies concerned with QS modeling also attempted to address QS in the context of biofilms.[24,105,106] Nilsson et al. for the first time asked the question how many bacterial cells constitute a "quorum" and demonstrated that compact organization of cells in the biofilm has a potential to result in the induction of the activated state even at relatively low cell density.[106] Koerber et al. constructed a reaction-diffusion model describing QS and formation of *P. aeruginosa* biofilm in burn wounds.[105] Explicitly introducing velocity of biofilm propagation due to the growth of its biomass, the same group later formulated a different mathematical approach to QS in growing biofilms by representing it as an advection-diffusion problem.[107] Extending established bioengineering approaches to modeling bacterial biofilms, Chopp et al. developed a 1D model of QS in *P. aeruginosa* biofilm.[108] Biological realism of their model was improved by taking into the consideration cell death and decay as well as oxygen consumption, factors that naturally limit the biomass growth. They concluded that bacteria, dwelling in anaerobic conditions close to the substratum and often considered metabolically dormant, should actively produce AI and reach the induced state first. In the follow-up study,[109] Chopp et al. extended and confirmed this theoretical prediction by imaging fluorescent QS reporter at various depths within the biofilm. They found localized clusters of induced bacteria in the immediate proximity to the underlying glass slide.

In realistic conditions, such as porous caverns in the soil or the lumen of a medical catheter, bacterial biofilms grow in the presence of a hydrodynamic flow which is likely to affect both the distribution of nutrients and the chemical communication among the bacteria. To address this important yet often ignored factor, Parsek, Chopp, and colleagues explicitly introduced advective flow into their models. In ref 110, they used the Stokes flow model to represent the flow of fluid above the biofilm and compared model predictions with the results of their experiments at different flow rates. As expected, introduction of even moderate flow significantly increased the cell density requirement to achieve quorum. Thus at the flow rate 0.04 mL/min (velocity 0.15 mm/s), the required density was 4×10^6 CFU/mm^2, while at 4.0 mL/min (15 mm/s), it was found to be 6×10^7 CFU/mm^2.

Further technological advance in modeling hydrodynamics of biofilms was reported by Duddu et al.,[111] where introduction of sophisticated numerical methods allowed the authors to consider complex profiles of cell density as well as detachment of biofilm fragments induced by the sheer stress. Armed with these tools, Chopp and colleagues recently revisited induction of QS in biofilms subjected to flow.[112] They found that, to make the situation even more complex, the influence of flow strongly depends on the morphology of the biofilm. Biofilms with rough, mushroom-shaped surface that are frequently found in experiments exhibit more complex dependence of the critical biomass on the flow rate and, generally require less biomass to reach quorum than their flat counterparts.

While continuous methods based on partial differential equations permit modeling large system sizes, they fail to capture discrete nature of biofilms composed of individual cells. Given the highly stochastic nature of the bacterial intracellular environment (see section 2.2), discrete methods, based on representation of cells as individual entities, may provide valuable complementary results. Proposed first in late 1990s[113,114] as simple cellular automata representing cells as nodes on a regular 2D lattice, discrete models have evolved into advanced tools with a potential to realistically simulate cell−cell communication in complex conditions of a biofilm. Thus, individual-based models developed by van Loosdrecht and colleagues[115−117] utilize spherical particles characterized by continuous location in 3D space and are able to independently grow, move, and divide. Rather than cell−cell communication, metabolic processes in biofilms, such as production of methane and nitrification, had been the primary focus of application of these methods. Agent-based methods, however, provide encapsulated sets of intracellular variables that can be used to simulate the dynamics of molecular networks, such as QSN, within individual cells. This approach was taken by Goryachev and colleagues in the development of their stochastic simulation environment in which cell agents could freely move and divide and had individual intracellular environments chemically connected to the outside medium.[37] Representation of cells as zero-dimensional points that can occupy the same or unrealistically close locations in the continuous 3D space, however, prevented this tool from being able to describe biofilm structure at a fine level of spatial resolution. More recently, agent-based methods were used by Xavier, Foster, and colleagues to address evolutionary aspects of cell−cell communication in biofilms.[118,119] In ref 119, they assumed that bacteria produce AI at a constant rate, and once the critical concentration is exceeded locally, some strains (termed QS+) terminate production of extracellular matrix (EPS) and instead invest all their resources into growth, while EPS+ and EPS− strains either produce EPS constitutively or do not produce it at all. They concluded that such quorum-sensing strategy will be evolutionary advantageous if biofilm dispersal is favored (the case of *V. cholera*) and detrimental in chronic, long-term biofilms (the case of *P. aeruginosa*). A platform for simulation of QS on a population scale but with a single cell resolution has also been reported by Melke et al., who combined intracellular gene-regulation dynamics and mechanical cell−cell and cell-wall interaction to model transition to quorum in various spatial layouts on the example of *V. fischeri*.[69]

5. Controlling Quorum Sensing to Reduce Bacterial Pathogenicity

Closely related to the field of quorum sensing in biofilms is the area of quorum sensing control in medical applications. Since the seminal work of Greenberg and colleagues on *P. aeruginosa*,[104] bacterial cell−cell communication has been progressively growing in importance as a potential target for anti-infective interventions.[120] Suppression of QS is advisible in two mutually nonexclusive situations, namely if: (1) in the quorum-induced state pathogenic bacteria produce biofilm that protects them from antibiotics and the immune system, (2) the state of quorum is required to mount aggressive virulence program. In the first scenario, reduction or complete destruction of autoinducer molecules can augment other, e.g., antibiotic, treatments, while in the second it may decrease pathogenicity without killing the microorganism, leaving this job to the immune system now relieved from the virulent attack. Thus a number of strategies, some of them based on the use of native autoinducer-degrading bacterial enzymes, have been proposed to fight pathogens of both animal[121,122] and plant[123,124] hosts.

Complementing these mostly experimental efforts, theoretical studies have also been conducted to identify optimal intervention targets and predict the therapy outcomes.[64,87,125−128] Thus, Fagerlind et al. suggested utilizing the property of many native QSNs to rapidly degrade the QSTR in the absence of the cognate AI by supplying cells with AHL antagonists.[129] Presumably, chemical compounds that strongly bind QSTR and block AHL-binding pocket without inducing active conformation of the transcription factor would suit the purpose. King, Ward, and colleagues utilized their population-based approach to QS[130] to study anti-AI strategies in both liquid culture and biofilms, predominantly on the example of *P. aeruginosa*[105,107,131−134] but more recently also in *S. aureus*.[135]

6. Cell−Cell Communication in Engineered Synthetic Systems

Synthetic biology, a novel branch of bioengineering, strives to develop standardized library of well-characterized regulatory elements to design modified microorganisms with desired properties.[13] Naturally, a module that can provide synchronization of gene expression across the whole population is a high-priority item on the list of synthetic elements. Because of their simple, well-characterized layout (see section 2.1), *luxR/I* type QSNs are promising candidates for such a module. Design and "debugging" of synthetic biology projects would hardly be possible without mathematical modeling that guides and supports the experimental effort throughout.

Among applications of QS in synthetic biology, population-wide synchronization of intracellular oscillators has captured perhaps the most attention. Collins and colleagues presented one of the first models predicting synchronization of synthetic intracellular oscillators by a *luxR/I*-type QSN.[136] Garcia-Ojalvo et al. constructed a model of an *E. coli* population modified by a "repressilator"-type oscillatory network whose individual intracellular dynamics was coupled across the population by a QS system.[137] In their model, they observed that individual cells were able to robustly synchronize into a collective rhythm despite intracellular noise. A number of theoretical studies further explored various aspects of oscillation synchronization by means of QS.[82,138−140]

Despite much invested effort, experimental realization of the proposed designs, however, has not been achieved until recently. At last, in early 2010, Hasty and colleagues reported the successful implementation of an engineered gene network that is capable of generating global oscillations in a growing population of cells.[141] In addition to the commonly used *V. fischeri luxI* and *luxR*, they utilized *aiiA* gene from *Bacillus thuringiensis* that encodes an AHL-degrading enzyme, lactonase. This gene was put under control of the *luxI* promoter, thus establishing a negative feedback loop that is necessary for oscillations. By using a microfluidic device, they manipulated the cell density and monitored the activity of a fluorescent reporter. Period and amplitude of observed synchronized oscillations were found to be dependent on the velocity of flow in the main channel of the microfluidic device. Interestingly, at low flow rates, they also detected striking waves of spatiotemporal activity propagating through the 100 μm chamber at velocities $\sim$8−35 μm per min.

Beyond synchronization of oscillations, QS-based coupling has been modeled and experimentally implemented in a number of engineered designs. Thus Kobayashi et al. implemented an *E. coli* strain that could activate transcription of any gene in a density-dependent manner by engineering a plasmid-born QS system based on the *V. fischeri luxR* and *luxI* genes.[13] Interestingly, by using a fluorescent reporter, they clearly observed bistability throughout the transition of their engineered QSN to the induced state. By further increasing complexity of the engineered behavior, Weiss and colleagues generated sender and receiver strains of *E. coli* so that the receiver cells responded to AI produced by the senders in a pulse-like manner.[142] Because the rate, with which the input signal increased, determined in this design the response amplitude, a number of interesting spatiotemporal patterns of induction has been observed. Specifically, the receivers were able to respond to rapidly increasing signal from the nearby senders and ignored slowly increasing signals from the farther located cells. In a subsequent paper,[143] Chen and Weiss demonstrated that, to establish a QS system, no bacterial components are absolutely necessary. Instead, they used budding yeast *S. cerevisiae* as a host that carried a plant (*Arabidopsis thaliana*) signaling system based on secretion and reception of a cytokinin, isopentenyladenine. This elegant and meticulous genetic-engineering task, which resulted in creation of a eukaryotic QSN entirely de novo, required the state-of-the-art experimental techniques and mechanistic knowledge accumulated by yeast genetics as well as careful planning involving substantial use of mathematical modeling.

If quorum-sensing allows bacteria to "measure" population density, can this information be fed back to, in fact, control the population size? Arnold and colleagues asked this question in a series of elegant synthetic biology studies and obtained positive answer. In ref 144, they inserted a toxin-encoding gene *ccdB* under the control of the *V. fischeri* QSN so that it was activated only at a sufficiently high cell density and observed that the engineered network indeed maintained cell density of the *E. coli* host at a controllable steady level. Assuming the existence of some time delay in this negative feedback system, it should be possible to observe at least transient oscillations. This indeed was reported by Balagadde et al., who cultivated the engineered strain in the microchemostat.[145] Although in nature QS is based on the autocrine signaling, theoretically nothing prevents a synergistic density-dependent action to be undertaken by two (or more) genetically distinct strains or even species. Brenner et al.[146] explored this possibility by engineering strains of *E. coli* harboring two distinct circuits so that circuit A expressed *P. aeruginosa lasI* and *rhlR*, while circuit B encoded *rhlI* and *lasR*. Thus, AI produced by strain A activated response in strain B and vice versa. As a result, mixed population consisting of both stains, named by the authors a microbial consensus consortium, exhibited transition to the induced state in a synergistic, mutually dependent manner. Finally, by crossing the QS-inducible toxin-mediated population control with the idea of cross-talking populations, Balagadde et al. generated a bacterial equivalent of the predator−prey ecosystem.[147] In this intricate design, the predator-produced AI (LasI generated 3oC12HSL) induced killing of the prey, while the prey-produced AI (LuxI generated 3oC6HSL) rescued the predator from expression of the toxin. The resulting synthetic ecosystem exhibited a variety of regimes including extinction of the prey, coexistence, and cell density oscillations. Another synthetic system with emergent oscillations was recently reported by Marguet et al. in *E. coli* strain engineered with a suicide circuit containing QS components and a lysis gene.[148]

7. Cell−Cell Communication beyond Quorum Sensing

While the majority of known bacterial cell−cell communication can be characterized as quorum sensing or, more generally, autoinduction,[98] a number of notable exceptions has also been described in the literature. In the Introduction, I defined QS as an (i) autocrine signaling system that (ii) regulates gene expression and relies on (iii) diffusive messengers. Violation of any or several of the above criteria renders the cell−cell communication system distinct from the classical "quorum sensing". Indeed, bacterial communication need not be always autocrine. The possibility of paracrine signaling emerges in bacterial species capable of differentiation into several distinct cell types, so that one cell type is a signal sender while another is a receiver. Indeed, Kolter and colleagues have recently described an example of such a communication in *B. subtilis*.[3] Formation of biofilm in *B. subtilis* is under control of two major signals: ComX and surfactin. ComX activates production of surfactin, while surfactin, in turn, induces production of extracellular matrix in a certain cell type. Cells that produce surfactin do not respond to it themselves. Moreover, it turns out that surfactin respondents become insensitive to ComX, thus precluding their differentiation into the surfactin producers.

In addition to, or instead of, altering the gene expression pattern, a signaling molecule can affect bacterial behavior through chemotaxis. Austin, Stock, and colleagues described a self-attractive mode of *E. coli* behavior[149,150] in which bacteria followed a chemotactic molecule, likely an amino acid, secreted by bacteria themselves. As a result, bacteria were found to accumulate in small confined spaces within micromanufactured chambers and mazes. Modeling of this behavior with Keller−Segel type reaction−diffusion equations fully reproduced their experimental observations.[149] Extending these results, Levchenko and colleagues constructed a microfluidic device to study formation of *E. coli* microcolonies in small confined spaces represented in their experiments by microfluidic chambers.[151] They demonstrated that the long-term growth in such environments can result in self-organized states with highly correlated organization of cells. With the help of in silico modeling, this effect was

attributed to the mechanical interaction between the cells and the chamber walls.

Another example of signaling with secreted amino acids has been reported by Salman and Libchaber, who found that direction of thermotaxis in *E. coli* is population-density-dependent.[152] Signaling molecule in this case is glycine that is both secreted and sensed by the cells, thus allowing bacteria to monitor the population density. Curiously, at low population density, bacteria swim toward higher temperature, but upon reaching a "quorum," they reverse the direction toward lower temperature. In both examples, signaling molecules affect chemotactic behavior rather then gene expression and the classical QS positive feedback loop is not closed because the signal is perceived but its production is not amplified. An interesting variation on the theme could be possibly observed if the chemotactic molecule was also a true quorum-sensing signal. Computational modeling suggests that in this case a spatially homogeneous bacterial population can spontaneously evolve into a stable pattern with multiple isolated maxima of cell density (Goryachev et al., unpublished results). While such a behavior as yet has not been reported in a wild-type bacterial population, potentially it could be achieved in a suitably engineered synthetic strain.

Finally, just like the cells of multicellular eukaryotes, bacteria can relay signals by direct physical contact of neighbors.[153] Perhaps the most studied example of this communication mode is the C-signaling in *Myxococcus xanthus*.[154] C-Signaling is mediated by a product of proteolytic cleavage of CsgA protein that is exposed on the outer membrane of *M. xanthus* and conveys information between two polarized cells aligned end-to-end. While the detailed biology of C-signaling and the associated multicellular development are beyond the scope of this review, it is noteworthy that mathematical modeling has been and is likely to continue playing a crucial role in understanding complex intra- and multicellular behavior of this extraordinary social microbe.[155−158]

8. Conclusions

Cell−cell communication among prokaryotes is an exciting multifaceted phenomenon that justly has attracted limelight in the past few decades. The field in itself has been a living testament to the systems biology cause by showing how experiment and theory can mutually enrich each other. Although the enormous progress in our understanding of the underlying molecular mechanisms and dynamic principles has been already achieved, much yet remains to be learned. Until recently, most of attention has been focused on the characterization of class A *luxR/I* quorum-sensing networks. Ample modeling literature proposed bistable, switch-like behavior of these QSNs in various Gram negative organisms. Yet, apart from a few notable examples described above, experimental validation of these predictions has been scarce. Outside of the class A QSNs, mechanistic knowledge is yet mostly fragmentary and our understanding of the governing principles is still far from complete. In particular, elusive operation principles of the class B *luxR/I* QSNs remain largely enigmatic. More work will be necessary also to better understand the integration of cell−cell communication into the overall bacterial decision-making machinery. Theoretical analysis and modeling have already proven their worth, yet their potential is far from being exhausted or even fully utilized. As follows from a number of recent successes, the best result is achieved when experimentation and modeling work closely together by iteratively informing and guiding each other. To achieve this, experimentalists and theoreticians should consciously reach for each other willing to break away from certain confounding dogmas and instead embrace the culture of their respective counterpart.[159]

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