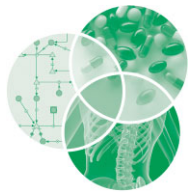
Advanced Review



Design principles of the bacterial quorum sensing gene networks

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Bacterial quorum sensing (QS) has attracted much interest as the manifestation of collective behavior in prokaryotic organisms once considered strictly solitary. Significant amount of genetic, biochemical, and structural data which, has been accumulated in studies on QS in many species allows us to map properties of specific molecules and their interactions on the observed population-wide bacterial behavior. The present review attempts to give a systems biology perspective on the structure of genetic regulatory networks that control QS and considers functional implications of a variety of design principles that recur in the organization of these networks across species. *WIREs Syst Biol Med* 2009 1 45–60

Quorum sensing (QS) refers to the ability of bacterial populations to coordinately regulate gene expression in response to changes in the local population density. Some examples of such collective behavior include secretion of antibiotics,[1–3] production of extracellular matrix,[4–6] emission of light,[7,8] and switch to swimming or swarming motility.[9–11] The cooperativity is achieved through the exchange of signaling molecules that are produced by individual bacterial cells and secreted in the surrounding extracellular space. QS therefore represents an example of a cooperative cell-cell communication[12] that occurs at a distance rather than through a direct physical contact of interacting cells.

Since the discovery of this phenomenon in marine luminescent bacteria *Vibrio fischeri*, a symbiont of a tropical squid, and its free-living relative *Vibrio harveyi*,[13–15] the QS research has evolved into a large and flourishing field that provided a totally new perspective on the general physiology, genetics, ecology, and pathogenicity of microorganisms. Indeed, the discovery of the QS promoted a dramatic shift in the microbiologist's perception of bacteria from dull solitary organisms incapable of any complex cooperative behavior to agile team players who continuously survey their environment for the presence of their kin, competitors, and enemies.

Comprehensive coverage of the vast field of QS, even in a succinct form, is far beyond the scope of this review. Historic perspective and modern views on the physiology and molecular biology of QS in individual bacterial species as well as extensive comparative analyses across multiple species can be found in many excellent reviews written by the pioneers of this field.[15–39] Burgeoning development in the past decade resulted in branching and diversification of the QS research. Thus, an unanticipated complexity of bacterial communication within the species, between close and distant taxons as well as with eukaryotic organisms (interkingdom signaling),[40] fostered the emergence of a new discipline of bacterial sociobiology that focuses on the evolutionary and ecological aspects of bacterial communication and cooperation.[39,41,42] Another newly formed branch studies QS from the perspective of the host-pathogen interactions[43–46] and seeks to develop novel antibacterial drugs that will interfere with QS and prevent or reduce bacterial pathogenicity toward animal[47–49] and plant[45,50] hosts.

The present contribution attempts to give a systems biology perspective on the intracellular mechanisms that regulate QS behavior on the molecular level. Unicellular nature, genetic tractability, and availability of complete genomic sequences have long placed bacterial regulatory networks into the focus of systems biology.[51–54] The QS phenomenon in particular provides an unprecedented opportunity to study molecular origins of collective multicellular behavior and perform comparative analyses of distinct network designs which evolved under the pressure of diverse ecological requirements.

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DOI: 10.1002/wsbm.027

WHAT IS 'QUORUM SENSING NETWORK'?

I refer to quorum sensing network (QSN) as a network of signaling and gene regulation events that are responsible for the production and perception of the signaling molecules as well as for the final outcome of communication—regulation of target genes whose products directly control specific functions, e.g., encode bacterial flagella. Depending on the complexity of the network, several layers of regulatory molecules may intercalate between the signaling molecules, also known as *autoinducers* (AIs), and the QS transcription factors (QSTRs) responsible for regulation of the target genes. Practically in all studied cases the QSN does not stand on its own but is instead imbedded into a complex regulatory network which integrates multiple environmental stimuli, such as pH, temperature, and the availability of nutrients.

Typically, the QSN consists of multiple components which can be placed into several functional categories. (1) Enzymes that produce signaling molecules, AI synthases, and the respective AI secretion systems. (2) QS sensor systems and transduction pathways that connect the receptors for signaling molecules to the regulators of QSTRs and possibly integrate other sensory inputs. (3) QSTRs and their direct regulators—proteins, RNA species, or metabolites that directly affect the activity of QSTRs themselves or regulate their transcription and translation.

QS SIGNALING MOLECULES, THEIR SYNTHASES AND SENSOR SYSTEMS

The choice of a signaling molecule, the physical messenger of cell–cell communication, imposes specific requirements on the presence and molecular properties of the components of the QSN that directly interact with the messenger. Indeed, membrane permeability, diffusivity, and stability to nonenzymatic degradation in the environment are all important biophysical properties of a signaling molecule that specify its signaling range and the means of its secretion and detection. Historically, two QS-signaling systems came to the limelight first: *N*-acylhomoserine lactones (AHLs) of Gram-negative bacteria[16,23,55,56] and peptides of Gram-positive species.[57–60] Early studies suggested that AHLs are low-molecular-weight metabolites that can passively diffuse through the cellular membranes. Many of these AIs were shown to bind directly to the QSTRs influencing their oligomerization state and affinity to the DNA *cis*-regulatory sequences.[55] Within this early conceptual model, AHLs require neither special secretory machinery nor cell-surface receptors. In contrast, peptides of Gram(+) bacteria are actively extruded from the cells and detected by transmembrane receptors[61] or actively imported back into the cells.[62,63] Further studies, however, reduced the divide between the two QS systems and identified a number of common features. Thus, it was shown that some AHLs with long acyl chains can hardly penetrate the membrane and thus require assisted transport.[64] Moreover, in some Gram(–) bacteria, e.g., *Vibrio* species,[35] AHLs are detected by cell-surface receptor kinases in a Gram(+) fashion.

AHLs are synthesized from metabolites readily produced in bacterial cells.[26,27] The best characterized family of AHL-synthases are homologs of *V. fischeri* LuxI, which are usually encoded in pairs with matching LuxR-type QSTRs.[16,65,66] Other, non-LuxI-type synthases, such as AinS (*V. fischeri*) and LuxM (*V. harveyi*),[67] with biochemical properties both similar and distinct from LuxI-type enzymes[56] have also been described. The length of the acyl chain is an important property of an AHL molecule. Indeed, chains shorter than four result in highly chemically unstable AHLs, which renders them generally unsuitable for cell–cell communication,[38] while AHLs with chains longer than 10 poorly diffuse through the cell membranes and might require transporters for their excretion.[64] Often, a single AHL synthase can produce a broad repertoire of AHLs with varying lengths of acyl chains.[68] Importantly, bacterial genomes also encode enzymes that degrade AHLs. Regulated activation of these enzymes, for example, in response to a stress signal ppGpp, can efficiently abrogate QS signaling.[69,70]

Peptide messages of Gram(+) species are genetically encoded as precursor peptides and do not require synthases *per se*.[60,61,71] Instead, these bacteria encode transmembrane enzymes, which proteolytically process the precursors while extruding them into the extracellular space. These proteins thus serve the function of synthases and exporters simultaneously. Two strategies are in use for detection of peptides in the environment: receptor histidine kinases[61] and active importers, usually from the family of ABC transporters.[62] Once imported into the cell, the peptides are sensed by intracellular receptors, such as *Bacillus subtilis* phosphatase Rap, whose activity is repressed by peptide binding,[72] thus preventing it from dephosphorylating a transcriptionally active response regulator.[62]

Both Gram-positive and Gram-negative species utilize a variety of furanones, collectively known as *AI-2*.[30] AI-2 molecules are a result of spontaneous cyclization of 4,5-dihydroxy-2,3-pentanedione (DPD), which together with homocysteine are the products of the LuxS group of enzymes first described

in *V. harveyi* and presently found in over 50 bacterial species.[21,30,73,74] Sensing of AI-2 as a QS signal has been studied in detail in *V. harveyi* and other marine vibrios in which AI-2 is bound by periplasmic protein LuxP and this complex is sensed by the transmembrane receptor LuxQ.[75] A different mode of interaction, which involves AI-2 internalization and degradation, has been described in enteric bacteria.[76] In these species, AI-2 is phosphorylated by intracellular kinase LsrK. AI-2-P then binds to and deactivates transcriptional repressor LsrR, which represses the *lsr* operon. First four genes of this operon (*lsrA–lsrD*) encode an AI-2-specific ABC importer, while products of the following genes, e.g., *lsrG*, are involved in the subsequent degradation of AI-2-P. Appearance of AI-2 in the extracellular medium thus first causes its low-level and possibly nonspecific internalization that activates the *lsr* operon, this in turn causes specific import and degradation of the messenger.

Expansion of the QS research resulted in the discovery of other signaling molecules with diverse chemical nature. In addition to AHLs, Gram(–) bacteria were also found to produce alkyl-quinolones,[77,78] DSF (*cis*-11-methyl-2-dodecenoic acid),[79] and fatty acid methyl esters.[80] Recently, the nature of an elusive *Vibrio cholera* AI CAI-1 has been identified and found to belong to yet another class of chemical compounds, 3-hydroxytridecan-4-one.[81] Not restricted to peptide signaling only, some Gram(+) bacteria, e.g., streptomycets, also synthesize γ-butyrolactones.[82] Future screens designed to identify bacterial cell–cell communication mediators will likely reveal additional signaling molecules.

NETWORK CORE: THE LUXR/LUXI PARADIGM IN GRAM-NEGATIVE BACTERIA

Already upon their original identification,[14] QSTR LuxR and the respective AI synthase LuxI were shown to be essential proteins for the QS regulation of luminescence in *V. fischeri*. A regulatory subnetwork based on the two genes, their protein products, and LuxI-generated AI rapidly became a paradigm for the QSN organization in Gram(–) bacteria.[16] Phylogenetic analyses of many genes encoding LuxR/LuxI homologs in various species[65] indicated that these genes coevolved as mutually regulating couples. This justifies their contemporary consideration as core regulatory modules of the AHL-based QSNs. Moreover, phylogenetic analysis shows that *luxR/luxI* homologs fall into two distinct classes.[83] Class A systems are widespread among various divisions of proteobacteria and their LuxR-type proteins are typically transcription activators. Class B systems, identified so far only in γ-proteobacteria, show a distinct pattern of genomic organization and their QSTRs are mostly repressors. LuxR-type QSTRs of both classes possess N-terminal AI-binding and C-terminal DNA-binding domains. Binding of cognate AIs to the LuxR-type proteins in stoichiometry 1 : 1 may result in either increase (class A) or attenuation (class B) of binding to the respective DNA *cis*-elements. Most of the class A QSTRs, such as the prototypical *V. fischeri* LuxR itself, are activators that require their cognate AIs for binding to DNA and transcriptional activation.[27,83] Competence for DNA binding in most of these QSTRs is correlated with their oligomerization state. Typically, these QSTRs are monomers in the inactive AHL-free state but dimerize efficiently in response to the AHL binding. This dimerization might be responsible for the observed protein half-life extension of the AHL-bound QSTRs.[84–86] Once dimeric, they strongly and specifically bind to the inverse-repeat DNA sequences of about 20 bp known as *lux-type boxes*.[16] Some exclusions from the above rules have also been reported. Thus *Erwinia caratovora* CarR was shown to be dimeric and competent to bind DNA already in the AHL-free state and formed higher-order oligomers in response to the AHL addition.[87]

More recently, a number of LuxR-type repressors, whose transcription repression is relieved by binding to AHLs, has been described.[88–92] Proteins of this type have been shown to be dimeric already in the AHL-free state,[90] which is consistent with their ability to bind palindromic DNA sequences and repress target genes in the absence of AHLs. Binding of AHLs to QSTRs triggers a conformational switch to the state that can no longer bind DNA. The complex releases DNA and transcription of target genes is activated. Interestingly, in both activator and repressor cases, accumulation of AIs effectively results in activation of the target genes.

While the ability of LuxR-type QSTRs to increase or reduce their affinity for DNA in response to AI binding appears to be a fundamental molecular property, their activator or repressor function should depend on the position of the respective DNA *cis*-element within the upstream regulatory sequence of a specific gene. Thus, conceivably the same QSTR could be activator for some and repressor for other target genes. Despite this theoretical possibility, class A QSTRs have been largely characterized as activators, while those of class B as repressors. ExpR1 and ExpR2 QSTRs recently characterized in *E. caratovora* may present an example of an alternative behavior.[93,94] In the AHL-free state, both proteins are DNA-bound and

possess other properties of class B QSTRs. However, they both activate transcription of regulatory mRNA-binding protein RsmA. 3-Oxo-C8-HSL selectively binds to ExpR2 and releases it from DNA, thus partially attenuating *rsmA* transcription. Another *E. caratovora*-produced AHL, 3-oxo-C6-HSL, binds to both QSTRs and releases them from DNA, further reducing *rsmA* transcription. Ironically, since RsmA is a repressor of the target genes (plant cell wall degrading enzymes), transition to the high-density state ('transition to quorum') results in the activation of target genes also in this case.

Importantly, *luxR/luxI* modules of different classes exhibit distinct genomic organization and regulatory interactions. Class A activators are presently understood the best. As shown schematically in Figure 1(a), a LuxR homolog is typically encoded separately from the operons it regulates and *luxI* is transcribed divergently or in the same direction as the *luxR* homolog. Normally, expression of the *luxI* homolog is positively regulated by the LuxR-type QSTRs.[16] Provided that LuxI-type synthases generate AI molecules necessary for the activity of the QSTRs, this activation of *luxI* transcription by LuxR constitutes the classical QS positive feedback loop that amplifies the intracellular production of AI in response to the extracellular AI. Although in this case production of the monomeric transcription factor remains at the same level, the copy number of the mature dimeric QSTR increases due to the increased formation of the LuxR-AI complex. Additional positive feedback loop may be provided by autoactivation of *luxR* transcription. This autoregulation can also amplify the copy number of the monomeric transcription factor and has been demonstrated at least for some QSTRs, e.g., for the prototypical *V. fischeri luxR*[95] and *E. caratovora carR*.[87]

In contrast, class B *luxR/luxI* modules of γ-proteobacteria exhibit strikingly different patterns of genomic organization and coregulation (Figure 1(b)). Similar to the described first *esaR/esaI* module,[88,90] other characterized representatives of this class (e.g., *expR/expI* of *E. chrysanthemi*[92] and *ypsR/ypsI* of *Y. pseudotuberculosis*[96,97]) are typically transcribed convergently with a small overlap between the transcribed frames of 20–25 bp, significance of which is not presently understood.[89,96] Unlike in class A, transcription of the *luxI* homolog generally is not directly regulated by the LuxR homolog and is controlled by other regulators.[97] As the QSTRs of this class are normally transcriptional repressors, autoinhibition of the *luxR* transcription is not uncommon but not obligate.[90,97]

In all known systems, mutual regulation within the *luxR/luxI* module accounts for only a part of the overall transcription regulation of the *luxR* and *luxI* homologs. Both may possess constitutive promoters as well as promoters regulated by nutrients,[98] signaling molecules,[99] and upstream transcription factors including other QSTRs.[97,100]

OTHER TYPES OF QSTRS

While characterized best, *luxR/luxI* paradigm does not exhaust all possibilities in bacterial QSNs. Even in Gram(–) species, *V. fischeri* LuxR homologs are not the only known QSTRs. Ironically, the best characterized examples of alternative QSTRs have been described in Vibrios, the same genus that gave birth to the *luxR/luxI* paradigm. Starting with the luminescence regulator LuxR in *V. harveyi*[101] (not related to *V. fischeri* LuxR, the protagonist of the LuxR class of QSTRs), a number of dimeric transcription factors that do not require AI molecules to bind to DNA were identified. Other representatives of this class of QSTRs are HapR in *V. cholera*[102–104] and LitR in *V. fischeri*.[105] Sequence and structure[106] homology indicate that these QSTRs belong to a large class of TetR transcription factors, dimeric proteins that bind palindromic *cis*-regulatory DNA sequences.[107] The protagonist of the class,

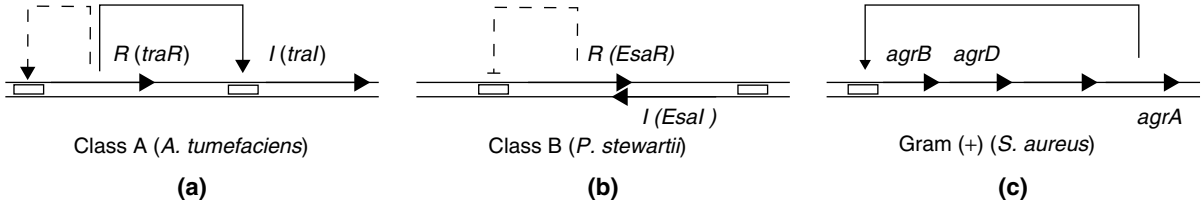

FIGURE 1 | Genomic organization and mutual regulation within the QSN core module. (a) Class A *luxR/luxI* module of Gram(–) bacteria. (b) Class B *luxR/luxI* module. (c) Representative example of Gram(+) QSN core module. Transcription regulator AgrA activates transcription of the *agr* operon. *agrD* and *agrB* encode AI peptide precursor and peptide extruding enzyme respectively. Two strands of DNA are represented by separate lines. Open boxes symbolize *cis*-regulatory elements. Regulatory interactions are shown by hammerheads (negative) and arrows (positive). Interactions found only in a subset of characterized systems are drawn by dashed line.

transcription repressor TetR controls the tetracycline exporter *tetA*, whose expression is activated when tetracycline binds to the TetR dimer and TetR dissociates from the *tetA* promoter.[107] Intriguingly, HapR, whose structure was recently determined,[106] also has a putative ligand-binding domain. Although TetR factors are generally considered as transcription repressors, their actual mode of action depends on the specific gene. Thus, HapR, most known for its repression of the *V. cholera* virulence master-regulator *aphA*, is also an activator of hemagglutinin HA/protease[102] and DNA uptake regulator.[108] As many other TetR transcription factors, HapR also represses its own expression.[109] The QS regulation of this class of QSTRs is believed to be performed by sRNA species, which effectively annihilate QSTR mRNA at low population density[110–112] (see a detailed discussion below).

QSTRs of Gram(+) bacteria, which are usually represented by the response regulators of two-component sensor systems, are typically found to positively regulate themselves and the ORFs encoding peptide precursors and their exporters.[61] This ensures that copy numbers of both the QSTRs and the signaling molecules are amplified in response to the QS signal. Genomic organization of Gram(+) QSN core modules is species specific.[58] The QSTR, signaling peptides, their receptors, and exporters may be encoded by the same operon, as in the case of *agr* operon of *Staphylococcus aureus*[60,113] (Figure 1(c)), or split into several operons co-regulated by the phosphorylated QSTR.[58,61]

IS THERE A SWITCH IN QSN AND WHAT FLIPS IT?

Principles that underlie operation of the QSN have captivated attention of researchers since the discovery of the QS phenomenon. The main function of the QSN, to control the copy number of the QSTR in response to the changing extracellular concentration of AI, can be achieved in two distinct ways. The QSN may operate as a rheostat by gradually changing the copy number of the QSTR and thus the expression of target genes. Alternatively, it may operate as a bistable switch with two distinct 'on' and 'off' states.[114,115] In the latter situation, an individual cell would be likely found in either uninduced or fully induced states, while cells with intermediate expression levels of the QSTR would be extremely rare.[116,117]

A well-known fact in mathematical sciences that positive feedback is a necessary condition for multistability[115,118] prompted a hypothesis that the core *luxR/luxI* module may, in fact, implement such a bistable switch. Assuming that the mature QSTR is a dimer that activates its own transcription, mathematical modeling of a *luxR/luxI*-type module demonstrated that, in principle, this network layout alone is sufficient to provide a bistable switching behavior.[119,120] Further analysis performed with realistic values of network reaction rates[121] identified a number of constraints imposed on this QSN layout and suggested its poor performance as a gene expression switch under the conditions of molecular noise.[122,123] Importantly, the *luxR/luxI* module may function as an autonomous, self-sufficient switch only if the QSTR is a relatively unstable dimer and the binding between the AI and the LuxR-type monomer is not very strong.[121] While this assumption implicitly made in Refs [119,120] appears to hold for some QS systems,[84,124] it is not universal for all *luxR/luxI* modules. Thus, *Agrobacterium tumefaciens* QSTR TraR forms practically irreversible complex with its cognate AI.[125] In fact, the molecule of AHL was found so deeply buried into the protein 3D structure that it was suggested that the complex forms only during TraR translation. Translated in the absence of AHL, TraR is misfolded, poorly soluble, and is rapidly degraded.[85,86,126]

Recent analyses of QS in *V. harveyi* and *V. cholera* suggested an alternative mechanism that can provide robust switchlike behavior without bistability.[110] In both species, AI signals are transmitted to the transcription regulator LuxO, which is phosphorylated in the absence of AIs (low cell density). Phosphorylated LuxO activates transcription of several Qrr small regulatory RNAs. In cooperation with chaperone Hfq,[127] these sRNAs form stable complexes with mRNAs of QSTRs LuxR (*V. harveyi*) or HapR (*V. cholera*). The Qrr sRNAs sequester QSTR mRNA and, either through the formation of an untranslatable complex or through the accelerated mRNA degradation, effectively prevent its translation. As opposed to repression by transcription factors, this type of post-transcriptional regulation results in the destruction of both the target (QSTR mRNA) and the regulator (Qrr sRNA) (Figure 2(a)). The target and regulatory RNAs mutually titrate each other and the outcome of their competition is completely defined by their respective copy numbers, which in turn sensitively depend on the rates of transcription. If transcription rate of either regulator or target (potentially both) depends on the concentration of AI, this mutually antagonistic interaction between RNA species may result in an ultrasensitive response[128,129] of the QSTR copy number to a gradual change in the AI concentration.[110] Thus, rapid, switchlike transition to quorum can be, in principle, achieved in a narrow

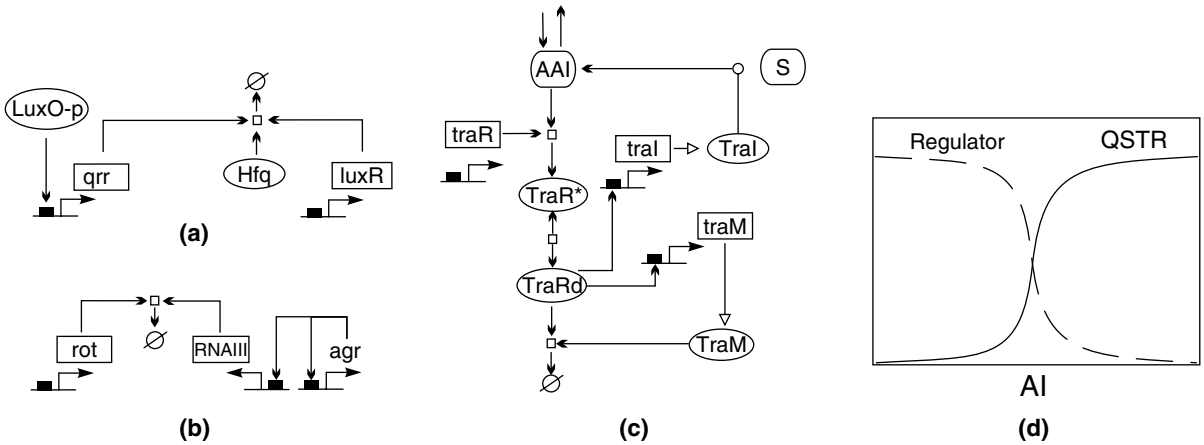

FIGURE 2 | Regulation of QSNs by mutual inhibition. (a) RNA–RNA switch in the QSN of *V. harveyi*. (b) RNA–RNA switch in the QSN of *S. aureus*. The QS positive feedback loop of *agr* operon is shown schematically. (c) Protein–protein mutual inhibition operates as a switch in *A. tumefaciens* QSN. AAI is Agrobacterium AI. S symbolizes substrates of AI synthase TraI. (d) Typical ultrasensitive behavior for expression of the QSTR and its inhibitory regulator schematically represented as a function of the extracellular concentration of AI. Small open boxes represent complex formation. Ø symbolizes eventual degradation of the inhibitory complex. RNA species are shown as rectangles and proteins as ovals. Translation of mRNA species is shown by open arrowheads.

range of changing concentration of the extracellular AI (schematically illustrated in Figure 2(d)).

The RNA-based mutual-antagonism switching mechanism has been also reported in other QSNs. Perhaps the oldest known example of this type of regulation is provided by RNAIII regulatory sRNA of *S. aureus agr* system.[130] RNAIII sequesters and inhibits translation of mRNAs for transcription factor Rot and protein A using almost the same mechanism as Vibrio Qrr sRNAs (Figure 2(b)). Presumably, activation of *agr* QSN network by its cognate AI peptide results in a similar ultrasensitive regulation of Rot. In addition, RNAIII forms an activatory complex with *hla* mRNA, which encodes α-hemolysin. Given that *hla* is repressed by Rot, the transition to quorum may result in a sharp switch between *rot* and *hla*.[58] Post-transcriptional control by small RNAs has recently become a major theme in prokaryotic gene regulation[131,132] and has attracted vivid interest among experimentalists and theoreticians alike.[133–136] Of particular prominence in the context of QSNs[137] is the regulatory motif based on the post-transcriptional regulator known as CsrA (in *E. coli*) or RsmA (in *Pseudomonas aeruginosa, E. caratovora*, and others) and the small RNA CsrB (RsmB).[132] The homodimeric CsrA/RsmA-type proteins mostly act as inhibitors of target genes by sequestering their mRNAs. Transcription of CsrB/RsmB sRNAs, which can bind simultaneously up to nine CsrA dimers,[132] results in fast and efficient sequestration of CsrA/RsmA repressors and activation of the target genes. In *E. caratovora*, repressor of plant cell wall degrading enzymes RsmA is under direct transcriptional control of QSTRs ExpR1 and ExpR2, and the respective sRNA RsmB is regulated by a GacS–GacA two-component phosphorelay activated in response to an as yet unknown extracellular signal.[138]

Mutual-antagonism switch of the QSN, however, does not have to depend on sRNAs. It has been long known that the functionality of *A. tumefaciens* QSN critically depends on the antiactivator TraM.[139,140] Knocking this protein out resulted in a constitutively high copy number of the QSTR TraR and a lockup of the network in the high-density, 'quorum', state. Recent work has demonstrated that TraM, whose expression is activated by the TraR dimer, is a homodimeric protein that forms highly stable protein complexes with TraR (two TraR dimers : two TraM dimers) and effectively inactivates the QSTR through the formation of this transcriptionally inactive complex[141] (Figure 2(c)). Mathematical analysis of the *A. tumefaciens* QSN[142] confirmed that TraR and TraM form a mutually antagonistic protein couple and demonstrated that they can produce an ultrasensitive switchlike transition to quorum in response to the AI signal (Figure 2(d)).

INTEGRATION OF QUORUM SENSING INTO GLOBAL GENE NETWORKS

Recent advance in understanding of bacterial gene networks demonstrated that the QSNs receive multiple regulatory inputs from various modules as well as co-regulate many groups of target genes

together with other networks and motifs.[143] The complexity of the regulatory cross-talk between the QSNs and other elements of the cellular control network varies from relatively low, such as in the well-understood case of *traR/traI* QSN that controls conjugal transfer of *A. tumefaciens* Ti plasmids,[98] to exceptionally high, such as in the QS circuitry of *P. aeruginosa*.[100,144,145]

If bacterium possesses several QSNs, they may cooperate following a variety of design patterns. Thus, several well-characterized examples of parallel QSN connection have been described in Vibrios.[35] In *V. harveyi*, at least three QS systems operate in parallel[111]: an AHL molecule HAI-1 and two interspecies signals CAI-1 and AI-2. The three signaling molecules are detected by their respective dedicated receptor histidine kinases LuxN, CqsS, and LuxQ, respectively. However, downstream of these receptors, the three signals come together as the common phosphotransferase protein LuxU collects the phosphates produced by the individual receptors and transfers them to the common response regulator LuxO, which in turn controls transcription of the small regulatory RNAs. Similar organization has been reported for *V. anguillarum* QSN,[146] while *V. cholera* seems to have two parallel QSN inputs mediated by generic signals CAI-1 and AI-2.[147] It has been reported that different AI inputs of the parallel QSN of *V. harveyi* translate into distinct levels of the master QSTR LuxR[148]; however, the mechanisms underlying this specificity are not yet completely understood.

A classical example of sequential arrangement of QSNs is provided by *P. aeruginosa* in which the *lasR/lasI* system has been shown to activate the *rhlR/rhlI* QS core module.[149,150] Another instructive example of hierarchical QSN organization has been described in *V. fischeri*, veteran of the QS field.[35] Recent research has demonstrated that, similar to other Vibrios, the sRNA-based *ain* QS system is the primary QSN also in this organism.[151] Receptor kinases detect at least two parallel inputs of the *ain* QSN—AinS-produced C8-HSL and AI-2—and through a LuxU-LuxO type phosphorelay control expression of LitR, a homologue of *V. harveyi* LuxR. LitR is thought to regulate the early phases of squid colonization, e.g., repress bacterial motility, and positively regulate the *luxR/luxI* QSN.[151,152] Full activation of the *luxR/luxI* QSN, which requires higher population density for the transition to quorum ($> 10^{10}$ cells/ml), results in the maximal induction of the *lux* operon as well as activation of at least 18 other genes.[153]

As microbiologists characterize more regulatory network motifs, it becomes increasingly clear that bacteria utilize essentially all of them in various combinations with the QSNs to achieve maximal flexibility in the control of gene expression. Rcs phosphorelay system,[154] described originally in *Escherichia coli*, provides an example of such a motif. Receptor kinase RcsC relays phosphate to the response regulator RcsB by means of the phosphotransfer protein RcsD. While some genes can be directly regulated by the RcsB homodimer, others require a cofactor RcsA, which forms a heterodimer with RcsB.[154] In *E. amylovora* and *Pantoea stewartii*, the *cps* operon responsible for the synthesis of capsule exoplysaccharide is under the control of the RcsA–RcsB heterodimer.[155] Recently, it has been shown that in *P. stewartii* QS controls capsule production through the regulation of RcsA.[4,156] In an ingenuous design, the prototypical repressor EsaR binds to a site between the weak constitutive promoter of RcsA and the strong autoinducible promoter, as shown in Figure 3(a). At high population density, the EsaR-mediated repression is relieved and massive quantities of RcsA are produced in a positive feedback loop.[156] As RcsA–RcsB also represses the flagella production, transition to quorum results in a switch between motile and sessile, capsule protected, life styles. Presumably, since RcsB is directly regulated by the uncharacterized ligand(s) of the RcsC sensor kinase, this integration design allows for the detection of coincidence (or logical AND operator) between QS and specific extracellular stimuli. This control mode, however, remains to be tested experimentally. Boolean AND operator between QS and another extracellular stimulus can be also achieved by hierarchical expression control as exemplified by *A. tumefaciens* QSN. The requirement for the presence of a specific plant-produced nutrient, octopine, is fulfilled by placing the QSTR *traR* within the opine-activated operon *occ*.[125]

Another motif frequently found together with QSNs is the RsmA/RsmB protein–RNA regulatory system, which has been already discussed in this review. In *E. caratovora*, sRNA RsmB is under the control of a GacS–GacA two-component system, while RsmA is positively regulated by ExpR1 and ExpR2.[93,94] When quorum is reached, both ExpR1 and ExpR2 are removed from the DNA and the expression of virulence factors, which were repressed in the absence of AHLs by RsmA, is activated. The same result could have been achieved, in principle, by an unidentified ligand of GacS through the activation of RsmB transcription. Thus, it can be hypothesized that the resulting QSN integration layout implements a logical OR operator allowing activation of the target genes either in response to the unknown signal or upon reaching quorum (Figure 3(b)).

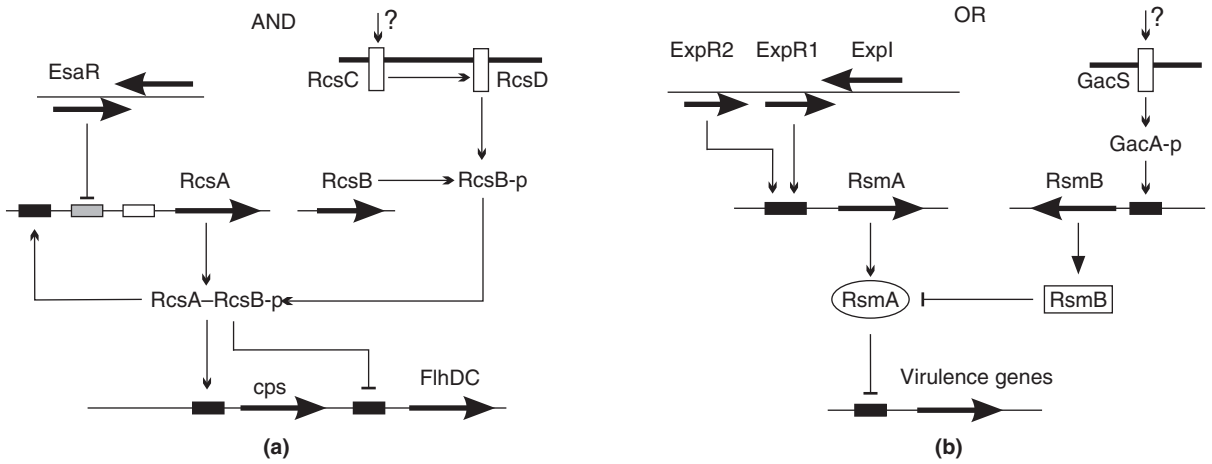

FIGURE 3 | Integration of quorum sensing with other sensory inputs. (a) Boolean AND logical circuit in the regulatory network of *P. stewartii*. (b) Boolean OR in *E. caratovora*. RNA species, except RsmB, are omitted for brevity. Notations are the same as in Figure 2.

A recent addition to the theme of signal integration is the recognition that the QSNs participate in the regulation of various cellular trends through the pervasive bacterial second messenger c-di-GMP.[157–159] Thus, c-di-GMP has been shown to positively regulate the formation of biofilm in a number of species including *V. cholera*.[160] In a recent study,[161] Bassler and colleagues demonstrated that in *V. cholera* the QSTR HapR regulates biofilm formation through two parallel pathways. Firstly, HapR binds to and directly represses the expression of the biofilm master regulator *vpsT*. In addition, HapR regulates a number of genes encoding proteins that generate and destroy c-di-GMP. The net result of this regulation is the decreased production of c-di-GMP in the state of quorum and, thus, downregulation of biofilm formation. While this exciting new story only begins to develop, it is already clear that QSNs, together with other extracellular-regulated networks, are involved in the c-di-GMP-dependent gene regulation in many bacterial species.[34,79,162–164]

CONCLUSION

QS gives bacteria a glimpse of the complex surrounding environment including the information on the presence of other cells of the same and distinct species. The QS detection mechanism is based on the production and reception of diffusible chemical molecules. Local concentration of the AI(s) in the immediate vicinity of the cell is the only actual extracellular input for the decision-making QSN. Given this information, a bacterium cannot distinguish between the situations when it is surrounded by a large number of other AI-producing cells or when it is enclosed alone in a small diffusion-impermeable compartment.[165,166] Owing to this inevitable ambiguity, the precise function of QS should be interpreted within the context of a specific ecological niche typical to the life style of the species in study.

Generalizing the design of a few currently well-characterized QSNs, it is tempting to hypothesize that all QSNs probably contain some form of a switch based on RNA–RNA, RNA–protein, or protein–protein mutual-antagonism interaction, which may or may not ensure the bistability of the whole network. The classical QS positive feedback loop implemented in all QSNs in one or another way amplifies the extracellular AI signal and likely trips the switch rather then encompasses the switch itself. Despite some theoretical arguments in favor of bistability, whether the QSNs are mostly 'rheostats' or 'switches' still remains to be determined experimentally. A recent test of this hypothesis in *V. harveyi*[148] resulted in a conclusion suggesting that its QSN operates in a 'rheostat' mode. However, the possibility that some of the QSNs exhibit genuinely bistable behavior still remains open.

QSNs are typically integrated with other bacterial sensory/decision-making networks to achieve precise and flexible control of gene expression. Several network motifs found ubiquitously across species enable bacteria to combine the QS signal with other signaling inputs using all three basic logic operators (AND, OR, and NOT), which are necessary to build Boolean decision rules of any desired degree of complexity. This opens a potential for cellular differentiation based on the spatial variation of environmental conditions throughout the population habitat, e.g. within a biofilm.[167–173] Indeed, the relationship between QS and bacterial multicellular

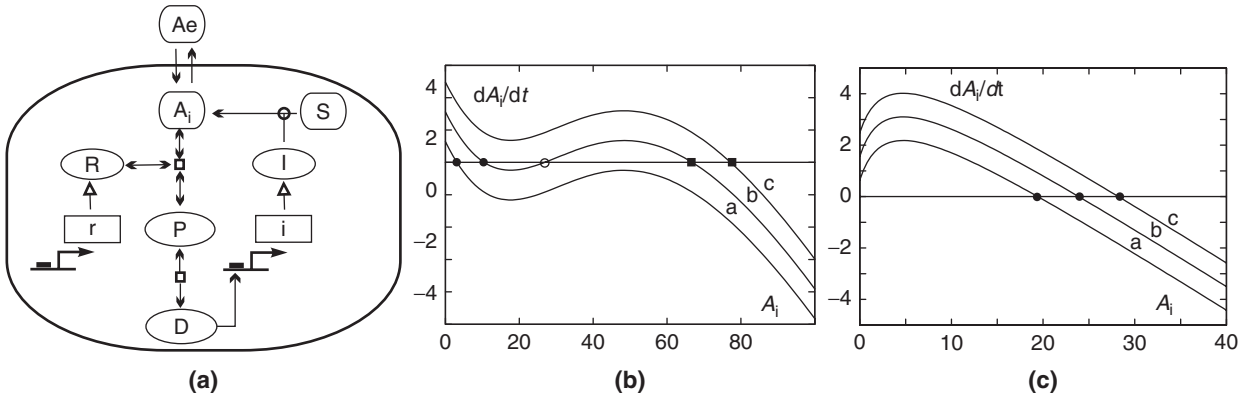

FIGURE 4 | Behavior of a hypothetical *luxR/luxI* type QSN. (a) Network layout. Notations are the same as in Figure 2. (b) Stationary states of the network at three values of extracellular AI: a—-0 nM; b—-12 nM; c—-24 nM. (c) After removal of the QSTR dimerization from the network layout, only one stationary state remains. (Same values of extracellular AI.) Filled circles and squares represent stable states of the network. Open circle denotes an unstable state of the bistable QSN. (Reaction rate values are adopted from Ref. 121).

morphogenesis has recently become an area of rapid expansion.[39,160,161,174–177] Future research will result in a major progress in understanding of the molecular mechanisms of QS and the connections between the QSN designs and the functional role of QS in the bacterial life style.

BOX1: MATHEMATICAL MODELING OF THE QSN DEMYSTIFIED

Mathematical modeling has become a powerful tool of biological research and its area of application continues to expand.[118,182] Even a relatively simple model that describes temporary dynamics of the QSN in terms of ordinary differential equations can shed light on the principles of its operation and reveal modes of behavior that are ***possible*** for a given network design. Conversely, it may also show that some experimentally observed behavior cannot be achieved by a hypothesized network layout and thus additional experimental work is warranted to identify missing or incorrectly characterized network elements.

To describe mathematically the dynamics of a hypothetical QSN shown in Figure 4(a) (generic layout of the *luxR/luxI* type module, adopted from Ref. [121]), one needs to describe how concentrations of the involved proteins, RNA species, and AI change in time. This can be done by using the mass-action rate law of chemical kinetics adapted to model complex cellular processes, such as transcription and translation.[119,120,183,184] For example, we can describe the rate of change for the concentration of luxR-like mRNA r as follows:

$$\frac{\mathrm{d}r}{\mathrm{d}t} = k_1 - k_2 r.$$

Here, according to the network layout, it is assumed that mRNA r is transcribed and degraded constitutively with rates k_1 and k_2 respectively. The mRNA r is then translated into a monomeric protein R, which reversibly binds a molecule of AI A (index i indicates intracellular concentration) to form complex P, which in turn reversibly dimerizes into a mature QSTR D. Describing QSTR-activated transcription of I (LuxI-like AI synthase), we assume that the RNA polymerase is abundant, while the availability of activator D is the rate limiting factor. However, I promoter has a maximal rate of transcription initiation and excess of D cannot speed up the transcription past this maximal rate. These requirements can be captured by the Michaelis–Menten kinetic law and the resulting rate of change for the mRNA concentration i becomes

$$\frac{\mathrm{d}i}{\mathrm{d}t} = \frac{k_3 D}{K_4 + D} - k_5 i.$$

Assuming abundance of ribosomes, translation of mRNA can be represented as a simple first-order reaction with a rate proportional to the mRNA concentration. Taking R protein concentration R as an example, we can describe the rate of R change as follows:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = k_6 r - k_7 R - k_8 R \cdot A_i + k_{-8} P.$$

The four terms in the above equation represent mRNA translation, protein degradation, formation of complex P, and its dissociation, respectively. Likewise, for the concentration of AI, which is continuously produced by LuxI, the rate of concentration change

is as follows:

$$\frac{dA_i}{dt} = k_{11}I - k_8R \cdot A_i + k_{-8}P + k_{14}(A_e - A_i),$$

where the last term represents passive diffusive exchange between intra- and extracellular molecules of AI. The latter serves as a free parameter of the model whose variation dictates the entire intracellular dynamics of the QSN.

Once the complete model is constructed (in this case, it consists of seven equations), it can be analyzed numerically on the computer. However, in some simple cases, like the example considered, the behavior of the model can be also studied analytically, e.g. by reducing the number of variables.[119,121,142] Thus, applying standard methods of chemical kinetics it can be shown[121] that dA_i/dt is a function of A_i, as shown in Figure 4(b) at three different values of A_e, the extracellular concentration of AI. Intersections of the function with zero axis symbolize stationary states of the QSN. The S-shaped form of the function ensures that at some intermediate concentration values of the extracellular AI the network is bistable. Indeed, it simultaneously has two steady states with low, corresponding to the 'off' state, and high, representing the 'on' state, concentrations of the intracellular AI. In biological terms, this mathematical result can be interpreted so that the given QSN layout ***may*** possess bistable switchlike behavior at some biologically plausible reaction parameters. If, however, we remove the dimerization of the QSTR from the reaction scheme in Figure 4(a) and instead assume that P is the transcriptionally competent QSTR, we will obtain a very different dependence of dA_i/dt on A_i (see Figure 4(c)). This modified QSN layout has a single steady state at any A_e and with any combination of model parameters. This implies that the variant of the QSN layout without QSTR dimerization ***cannot*** be a 'switch' in a strict sense but instead will always behave as a 'rheostat'.

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FURTHER READING

Quorum sensing represents an example of bacterial cell–cell communication. A generally accessible and concise introduction to this topic can be found in the enlightening and visually appealing review[178] by Losick and Kaiser. A systematic account of this field, including quorum sensing, is compiled in the comprehensive collection edited by Dunny and Winans.[12] Because communication is crucial for complex organization, bacterial multicellular morphogenesis is another exciting topic related to quorum sensing.[179–181] Those interested in application of mathematical modeling to the analysis of QSN are referred to the clear and accessible paper by Dockery and Keener.[119] Further references on modeling and its role in understanding intracellular decision circuitry can be found in the excellent reviews written by the pioneers of this field.[114,115,118,182]

