Review TRENDS in Cell Biology Vol.14 No.11 November 2004

Bacterial social engagements

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Quorum sensing is a process that enables bacteria to communicate using secreted signaling molecules called autoinducers. This process enables a population of bacteria to regulate gene expression collectively and, therefore, control behavior on a community-wide scale. Quorum sensing is widespread in the bacterial world and, generally, processes controlled by quorum sensing are unproductive when undertaken by an individual bacterium but become effective when undertaken by the group. Cell–cell communication can occur within and between bacterial species, and between bacteria and their eukaryotic hosts, which suggests that the chemical lexicon is complex. Prokaryotic and eukaryotic mechanisms for enhancing and inhibiting quorum sensing have been identified, which suggests that manipulation of quorum-sensing-controlled processes could be common in bacterial–bacterial and bacterial–eukaryotic associations.

Using a process called quorum sensing, bacteria regulate gene expression in response to changes in cell-population density. Quorum sensing involves the production, release and subsequent detection of chemical signaling molecules called autoinducers. As a population of autoinducer-producing bacteria grows, the extracellular concentration of autoinducer increases with increasing cell number. When the autoinducer reaches a crucial threshold level, the group responds with a population-wide alteration in gene expression. Linking alterations in gene expression to the presence of autoinducer gives bacteria a means to perform specific behaviors only when living in a community, not when living in isolation. Behaviors controlled by quorum sensing are usually ones that are productive only when carried out simultaneously by many cells. For example, quorum sensing controls secretion of virulence factors, formation of biofilms, conjugation, sporulation and bioluminescence [1]. In this article, we focus on mechanisms of quorum-sensing signal production, detection and relay. We also describe new evidence suggesting that, in symbiotic and pathogenic relationships between bacteria and eukaryotes, the eukaryotic hosts often detect and respond to bacterial quorum-sensing signals.

Canonical quorum-sensing systems

Quorum-sensing systems can be divided into three primary classes based on the type of autoinducer signal and the apparatus used for its detection. First, Gram-negative bacteria typically have LuxI/R quorum-sensing systems [2,3] (Figure 1, left column). LuxI-type enzymes synthesize acylated homoserine lactone (AHL) autoinducers by ligating a specific acyl moiety from an acyl–acyl carrier protein to the homocysteine moiety of *S*-adenosylmethionine (SAM) [4,5]. The LuxR-type proteins bind their cognate autoinducers and control transcription of target genes. LuxI/R systems have been identified in over 70 species of Gram-negative bacteria [1,6].

The second class of quorum-sensing system is in Gram-positive bacteria, which use modified oligopeptides as autoinducers. The signals are synthesized as precursor peptides, which are subsequently processed and secreted [7,8]. Often oligopeptide autoinducers contain side-chain modifications such as isoprenyl groups (*Bacillus subtilis*) or thio-lactone rings (*Staphylococcus* spp.) [9–11] (Figure 1, center column). Two-component signal transduction proteins called sensor histidine kinases detect the extracellular peptide autoinducers, autophosphorylate, and transmit sensory information via phosphorylation of a two-component response regulator protein [12]. Phosphorylation of the response regulator modifies its DNA binding activity, and enables it to control transcription of quorum-sensing target genes.

Bacteria that use LuxI/R and oligopeptide–two-component quorum-sensing systems use them primarily for intraspecies cell–cell communication. Each bacterial species produces a signal that differs from that produced by most other species, and the LuxR-type and two-component receptors are extremely sensitive to the structures of their cognate autoinducers. Thus, minor modifications in the autoinducers often abolish detection by the sensor.

A third class of quorum-sensing system is a hybrid between the canonical Gram-negative and Gram-positive systems (Figure 1, right column). This hybrid system was initially identified in the bioluminescent marine bacterium *Vibrio harveyi*, which produces and detects two distinct autoinducers, AI-1 and AI-2 [13,14]. Similar to other Gram-negative systems, AI-1 is an AHL [15]. By contrast, AI-2 of *V. harveyi* is a furanosyl borate diester with no resemblance to other autoinducers [16]. As in Gram-positive systems, AI-1 and AI-2 signal transduction occurs by a two-component phosphorylation cascade. Additional response regulator and HPt (histidine phosphotransferase) modules, not included in the canonical Gram-positive circuit (Figure 1), are in the *V. harveyi* signal relay. Incorporation of several modules into two-component signaling cascades is not an uncommon arrangement.

V. harveyi AI-1 activity is only known to be produced by the closely related species *Vibrio parahaemolyticus* suggesting that, like other AHL-type signals, AI-1 is used for intraspecies communication [17]. By contrast, AI-2 and its synthase, LuxS, are widespread, existing in many

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Available online 12 October 2004

 0962-8924/$ - see front matter doi:10.1016/j.tcb.2004.09.012

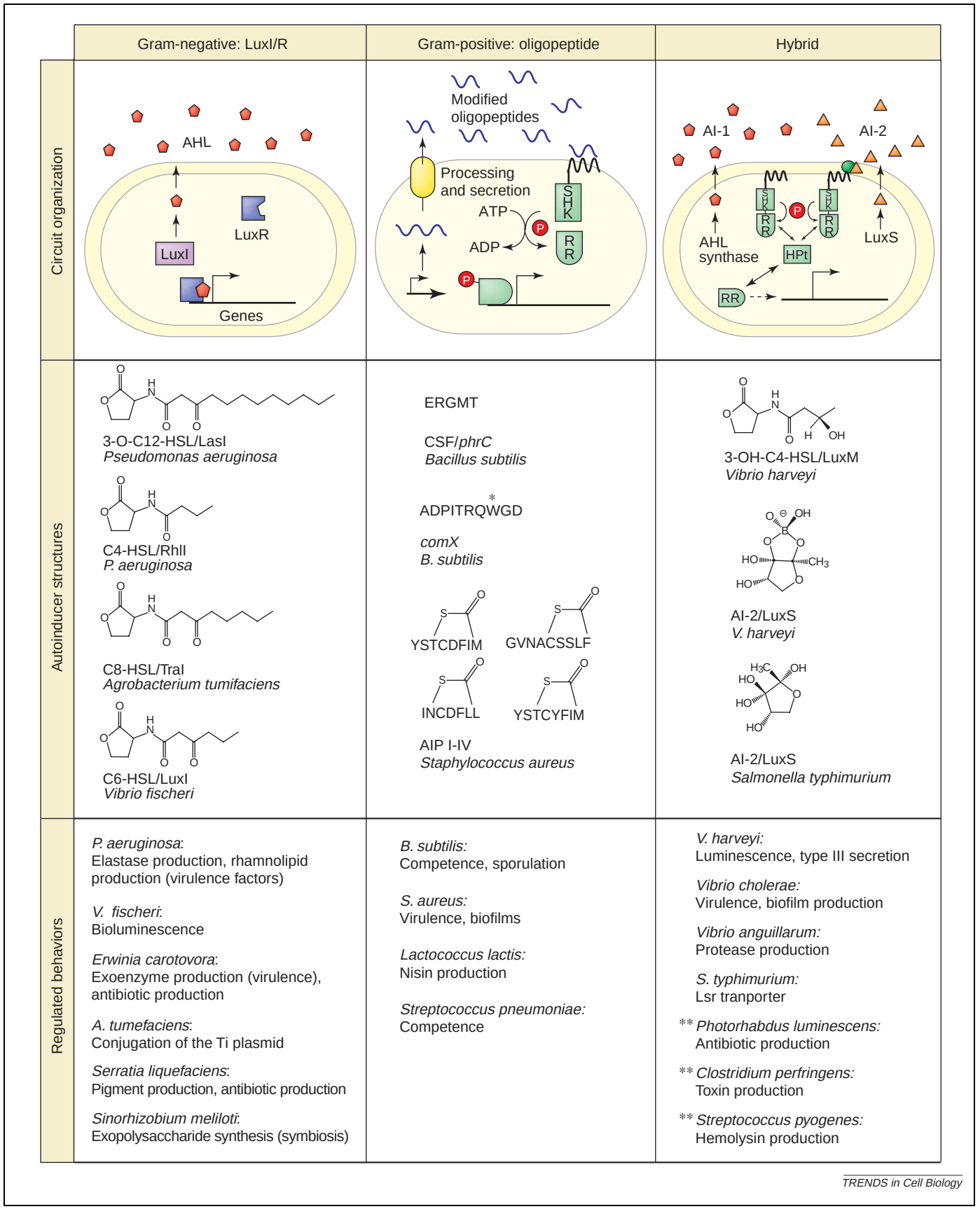

Figure 1. Canonical quorum-sensing systems. The three general classes of quorum-sensing systems are: Gram-negative LuxI/R-type (left column), Gram-positive oligopeptide–two-component-type (middle column), and a hybrid type that has features of both Gram-negative- and Gram-positive-type systems (right column). Red pentagons denote acylated homoserine lactones (AHLs); wavy blue lines denote oligopeptides; orange triangles denote autoinducer-2 (AI-2). Representative autoinducers and quorum-sensing-controlled behaviors are shown. A single asterisk denotes an isoprenyl modification. Bacterial species are marked with a double asterisk if the mechanism of AI-2 detection is not known [41]. Abbreviations: HPt, histidine phosphotransfer protein; P, phosphate; RR, response regulator; SHK, sensor histidine kinase.

bacterial phyla. This broad distribution, coupled with the finding that AI-2 is produced and detected by a variety of bacteria, suggests that AI-2 could serve as an interspecies bacterial communication signal [17,18].

AI-2 is produced from SAM in three enzymatic steps (Figure 2a). When SAM is used as a methyl donor, *S*-adenosylhomocysteine (SAH) is produced. The enzyme Pfs converts SAH to *S*-ribosyl homocysteine (SRH) and adenine, and subsequently LuxS acts on SRH to make homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD) [19]. DPD is the core molecule from which all AI-2s are derived. The structures of two biologically active AI-2 molecules are currently known. In *V. harveyi*, DPD cyclizes, is hydrated, and is converted to the final AI-2 signaling molecule by the addition of borate and the loss of water (Figure 2b, upper pathway) [16]. In *Salmonella typhimurium*, active AI-2 is the hydrated form of DPD that has cyclized with stereochemistry opposite to that of *V. harveyi* AI-2 (Figure 2b, lower pathway). Interestingly, the *V. harveyi* and *S. typhimurium* AI-2s undergo interconversion, revealing new complexity in bacterial interspecies chemical communication [20].

Signal discrimination and information integration

Many bacteria possess multiple quorum-sensing systems, which can be organized in series or in parallel. In *V. harveyi*, the quorum-sensing systems function in parallel and converge to regulate a common set of target genes [21]. *B. subtilis* also uses parallel systems to respond to different oligopeptide autoinducers that control distinct groups of downstream genes [22]. In contrast to these arrangements, *Pseudomonas aeruginosa* uses two LuxI/R systems (called LasI/R and RhlI/R) acting in series to regulate overlapping groups of target genes [23].

The apparent complexity of bacterial chemical communication systems has led to questions concerning how bacteria distinguish multiple temporally coincident chemical cues, how they preserve the information encoded in each signal, and how and if they integrate the information from multiple signals. Although analyses aimed at deciphering the complexity of these communication circuits are only in the early stages, some preliminary understanding has come from studies of *V. harveyi* and *B. subtilis*.

Vibrio harveyi *and coincidence detection*

V. harveyi uses a 'coincidence detection' mechanism to respond to its multiple autoinducers [21]. As mentioned, *V. harveyi* produces and detects two autoinducers called AI-1 and AI-2, which are detected by distinct sensor histidine kinases, LuxN and LuxQ, respectively [13,14] (Figure 3). LuxQ works in conjunction with a periplasmic

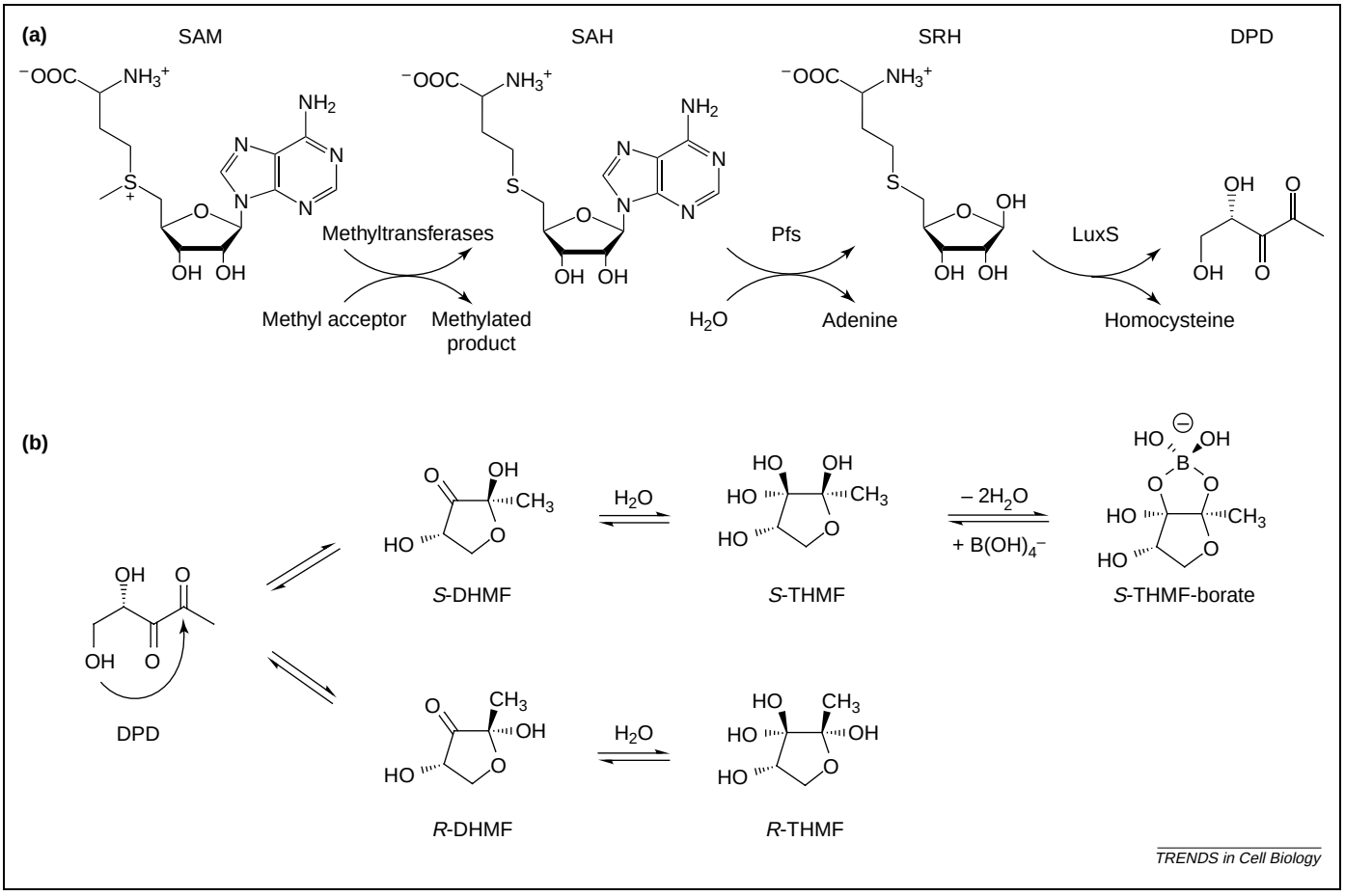

Figure 2. Biosynthesis of autoinducer-2 (AI-2). (**a**) 4,5-Dihydroxy-2,3-pentanedione (DPD), the precursor to all AI-2s, is synthesized from *S*-adenosylmethionine (SAM) in three enzymatic steps. (**b**) DPD rearranges and undergoes further reactions to form distinct biologically active signal molecules that are generically termed AI-2. The *Vibrio harveyi* AI-2 (*S*-THMF-borate) is produced by the upper pathway, and the *Salmonella typhimurium* AI-2 (*R*-THMF) is produced by the lower pathway. Adapted, with permission, from Ref. [20].

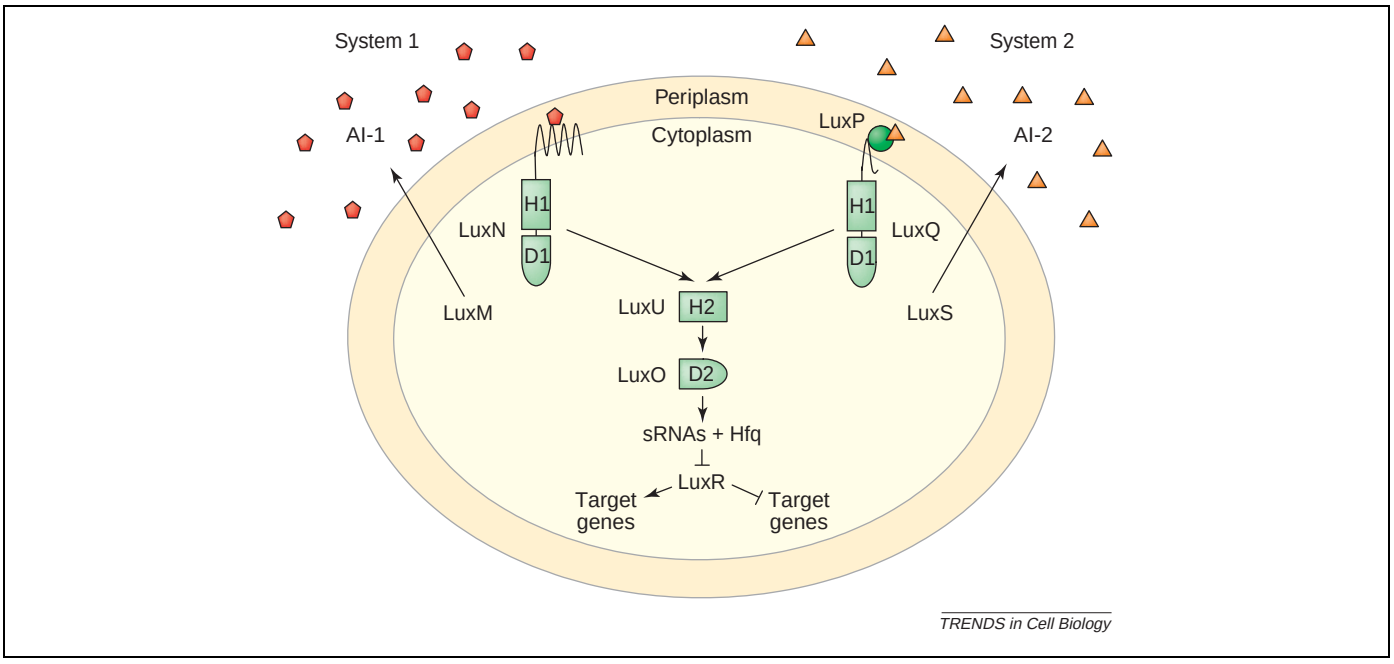

Figure 3. The *Vibrio harveyi* quorum-sensing system. Two quorum-sensing systems function in parallel to regulate gene expression in *V. harveyi*. Red pentagons and orange triangles represent autoinducers 1 and 2 (AI-1 and AI-2), respectively. Phosphate flows in the direction indicated by the arrows at low cell density (towards LuxO) and in the opposite direction at high cell density (phosphate is drained from LuxO). Phospho–LuxO activates the transcription of multiple small regulatory RNAs (sRNAs) that, together with the RNA chaperone Hfq, destabilize the *luxR* mRNA. LuxR is the master transcriptional regulator of the quorum-sensing cascade. H and D denote histidine and aspartate, respectively. These residues are the sites of phosphorylation.

AI-2 binding protein called LuxP. Following detection, the information contained in the two signals is integrated: both LuxN and LuxQ transfer phosphate to the same histidine phosphotransfer protein called LuxU. LuxU, in turn, transmits the phosphate signal to the response regulator LuxO. Phospho-LuxO is active and controls downstream gene expression. Thus, sensory information stemming from LuxN and LuxQ converges onto LuxO [24].

In *V. harveyi* there are four possible input states: no autoinducer, AI-1 only, AI-2 only, or AI-1 and AI-2. Interestingly, significant alterations in quorum-sensing-controlled target gene expression occur only when both autoinducers are present concurrently. This observation implies that the *V. harveyi* circuit primarily distinguishes between the simultaneous presence of both autoinducers (AI-1 and AI-2) and all other conditions (no autoinducer, AI-1 only, and AI-2 only), leading to the proposal that the circuit functions as a 'coincidence detector' [21]. A possible benefit of using a coincidence detection scheme is that sampling the environment for two signals reduces the sensitivity of the system to noise or to 'trickery' from bacteria that produce molecules mimicking the *bona fide* autoinducers (Box 1). 'Coincidence detectors' also exist in eukaryotes – in neocortical pyramidal neurons, auditory fibers and electrical synapses – and, similar to the case of autoinduction, they integrate sensory information from distinct sensors [25–27].

Bacillus subtilis *and signal antagonism*

B. subtilis has adopted a different strategy from *V. harveyi* for responding to multiple peptide autoinducers. In *B. subtilis*, as in *V. harveyi*, the different autoinducer inputs impinge on the same response regulator. However, in contrast to *V. harveyi*, in which information from one signal reinforces the information sent from the other signal, in *B. subtilis* the signals have opposing effects on the response regulator (i.e. one causes phosphorylation and one causes dephosphorylation). This strategy lets *B. subtilis* opt between one of two mutually exclusive lifestyles, competence (the ability to take up exogenous DNA) and sporulation (Figure 4).

In *B. subtilis*, the modified oligopeptide, ComX, and its two-component sensor, ComP, are required for competence. Binding of ComX causes ComP to autophosphorylate and transfer phosphate to the response regulator, ComA [28,29]. Phospho-ComA activates transcription of genes required for development of the competent state [30]. CSF, a second secreted peptide autoinducer, at high concentrations antagonizes this process. Unlike ComX, CSF is transported back into the bacterial cell by an oligopeptide permease (Opp). Intracellular CSF inhibits the ComP–ComA signaling cascade, and inhibits the phosphatases RapA, RapB and RapE that dephosphorylate the response regulator called Spo0F following its phosphorylation by three cognate kinases, KinA, KinB and KinC [31–33]. This latter inhibition leads to increased phospho-Spo0F – a species required to induce expression of genes necessary for sporulation (Figure 4). Thus, the combined inactivation of the ComP–ComA signaling cascade and the activation of Spo0F caused by CSF favors the development of spores over competence.

Fidelity of autoinducer responses

The transition from acting as an individual to participating in a collective activity is a crucial one for bacteria. It is therefore not surprising that these alterations in behavior

Box 1. Interference with quorum sensing

Because quorum sensing controls fundamental processes involved with both bacterial physiology and virulence, it is not surprising that prokaryotes and eukaryotes have evolved strategies to interfere with quorum sensing. Autoinducer antagonists, autoinducer destroying enzymes, and other mechanisms for consuming autoinducers are now known to enable 'quorum quenching' [56]. A remarkable example of intraspecies bacterial–bacterial quorum quenching is observed in the human pathogen *Staphylococcus aureus*. *S. aureus* strains are classified according to the sequence of their autoinducing peptides. Each peptide activates quorum-sensing-controlled gene expression within its group and inhibits quorum-sensing behaviors in all the other groups [57]. Interspecies quorum quenching has been established in some *Bacillus* species. These use peptides as autoinducers, and some also make lactonase enzymes such as AiiA that hydrolyze the lactone ring of acylated homoserine lactones (AHLs), greatly reducing their signaling activity [56,58]. Quorum-sensing-controlled behaviors of *Bacillus* spp. are not affected by this quenching strategy because AHLs, not peptides, are the communication signals targeted for sabotage. Other AHL-destroying enzymes exist. AiiD, from *Ralstonia* species, is an acylase that inactivates AHLs by hydrolyzing the AHL–amide bond [59].

In a case of eukaryote–prokaryote quorum-quenching, the seaweed *Delisea pulchra* makes halogenated furanones that inhibit swarming in *Serratia liquefaciens* and inhibit biofilm development by *Pseudomonas aeruginosa*, both quorum-sensing-controlled behaviors [60]. Halogenated furanones are structural analogs of AHLs, and function by reducing the half-life of LuxR type proteins [61]. However, the halogenated furanones also inhibit quorum-sensing behaviors in bacteria without LuxI/R quorum-sensing systems, suggesting that the furanones might interfere with several classes of autoinducer signals. Finally, recent work shows that quorum-quenching processes could exist in humans, because human airway epithelial cells possess a membrane-associated activity that destroys *P. aeruginosa* 3-O-C12-HSL but not C4-HSL [62].

Quorum-quenching studies have only recently been undertaken. However, these initial studies suggest that many different mechanisms for interfering with chemical communication between bacteria exist and await identification. These studies also highlight novel strategies that might be developed in the biotechnology industry for controlling bacterial infections. Advances have been made in implementing quorum-quenching strategies for biotechnological purposes. Transgenic potato and tobacco plants expressing the *Bacillus aiiA* gene encoding the AHL lactonase have enhanced resistance to *Erwinia carotovora* infections because *E. carotovora* uses AHL quorum sensing to control virulence factors required for disease in plants [56]. Heterologous expression of *aiiD* in *P. aeruginosa* eliminates AHL-dependent quorum-sensing behaviors like virulence and biofilm formation [59]. Introduction of synthetically produced quorum-quenching halogenated furanones into mice followed by exposure to *P. aeruginosa* attenuates virulence and increases the survival of the mice [63].

are highly regulated and many conditions must be met before a bacterium switches from low-cell-density to high-cell-density mode. Bacteria have placed sophisticated regulatory devices at different locations in quorum-sensing signal-transduction cascades to guarantee that this transition occurs under the appropriate set of circumstances and with high reliability. We describe a few of these high-fidelity apparatuses here.

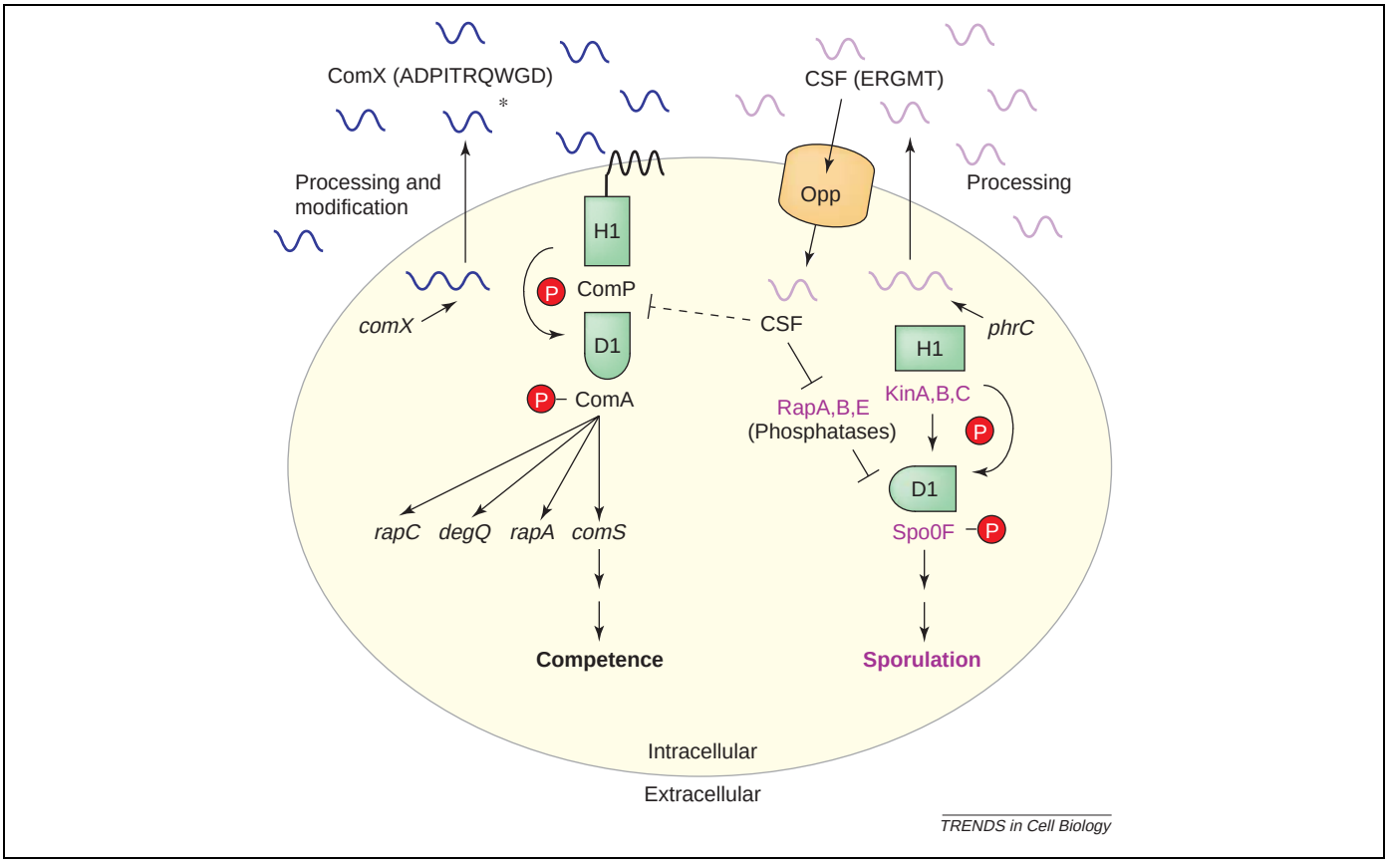

Figure 4. The *Bacillus subtilis* quorum-sensing system. Extracellular and intracellular oligopeptides control competence and sporulation, respectively. Competence: the peptide autoinducer ComX (blue wavy lines) contains an isoprenyl modification (asterisk) on a tryptophan residue. Sporulation: *phrC* encodes the precursor to the pentapeptide autoinducer CSF (purple wavy lines). ComX functions from the outside and CSF functions from inside the cell. The oligopeptide permease (Opp) transporter imports CSF. The peptides cause changes in the levels of phospho-ComA and phospho-Spo0F, which, in turn, regulate genes required for competence and sporulation, respectively. Broken line represents uncharacterized interactions; H and D denote histidine and aspartate sites of phosphorylation, respectively. Abbreviation: P, phosphate.

Pseudomonas aeruginosa *and temporal ordering of quorum-sensing responses*

As mentioned, the Gram-negative opportunistic pathogen *P. aeruginosa* has two LuxI/R-type quorum-sensing systems that function in tandem to control a variety of virulence factors (Figure 5). The first system, Las, consists of LasI, which produces 3-O-C12-HSL (homoserine lactone), and its cognate autoinducer-binding transcriptional activator protein, LasR [34–36]. The second system, Rhl, is made up of RhlI and RhlR and the autoinducer is C4-HSL [37]. The LasI-produced autoinducer, 3-O-C12-HSL, accumulates first and induces LasR to upregulate the expression of several downstream target genes, one of which is *lasI* [38]. This positive feedback step increases LasI production, and in turn, increases 3-O-C12-HSL production (Figure 5). Flooding the environment with autoinducer probably ensures that once the first few bacteria have committed to quorum-sensing mode, the remainder of the population follows. Another gene controlled by the LasI–LasR circuit is *rhlI*, encoding the C4-HSL synthase [39]. RhlR binds C4-HSL and activates its set of target outputs. The Las and Rhl quorum-sensing systems regulate partially overlapping sets of target genes. This tandem mechanism guarantees that the targets under RhlI/R control are turned on after the LasI/R controlled targets, which could be important for the proper development of *P. aeruginosa* biofilms or for sequentially turning on genes that promote early and late events in the infection process.

Vibrio harveyi *and ultrasensitivity*

An ultrasensitive switchlike mechanism is used by *V. harveyi* and *V. cholerae* to control the 'all-or-none' transition from low-cell-density-mode to high-cell-density, quorum-sensing mode [40]. The *V. harveyi* and *V. cholerae* quorum-sensing circuits are extremely similar, and one of the regulatory components that both bacteria possess, LuxO, is phosphorylated at low cell density. Phospho-LuxO activates the expression of genes encoding four homologous small regulatory RNAs (sRNAs) in *V. cholerae* and probably five in *V. harveyi* (Figure 3). These sRNAs function in concert with the chaperone Hfq to destabilize the mRNA encoding the master regulator of the quorum-sensing cascade (*hapR* in *V. cholera*e, *luxR* in *V. harveyi*; note the *V. harveyi* LuxR is not similar to LuxRs of the LuxI/R type). Hfq fosters base pairing between the sRNA and the target mRNA, which promotes the degradation of both the sRNA and the mRNA. Surprisingly, although multiple homologous sRNAs are involved, any one of them is sufficient for complete quorum-sensing repression [40]. Control via sRNAs is hypothesized to enable an ultrasensitive (switchlike) response to the level of phospho-LuxO. Because base pairing between the sRNA and the mRNA promotes their mutual destruction, if the rate of synthesis of an sRNA exceeds the rate of synthesis of the target message, even if only slightly, then the sRNA will accumulate and the target mRNA will be reduced to negligible levels. By contrast, if the rate of synthesis of the target mRNA exceeds that of its regulatory sRNA, then

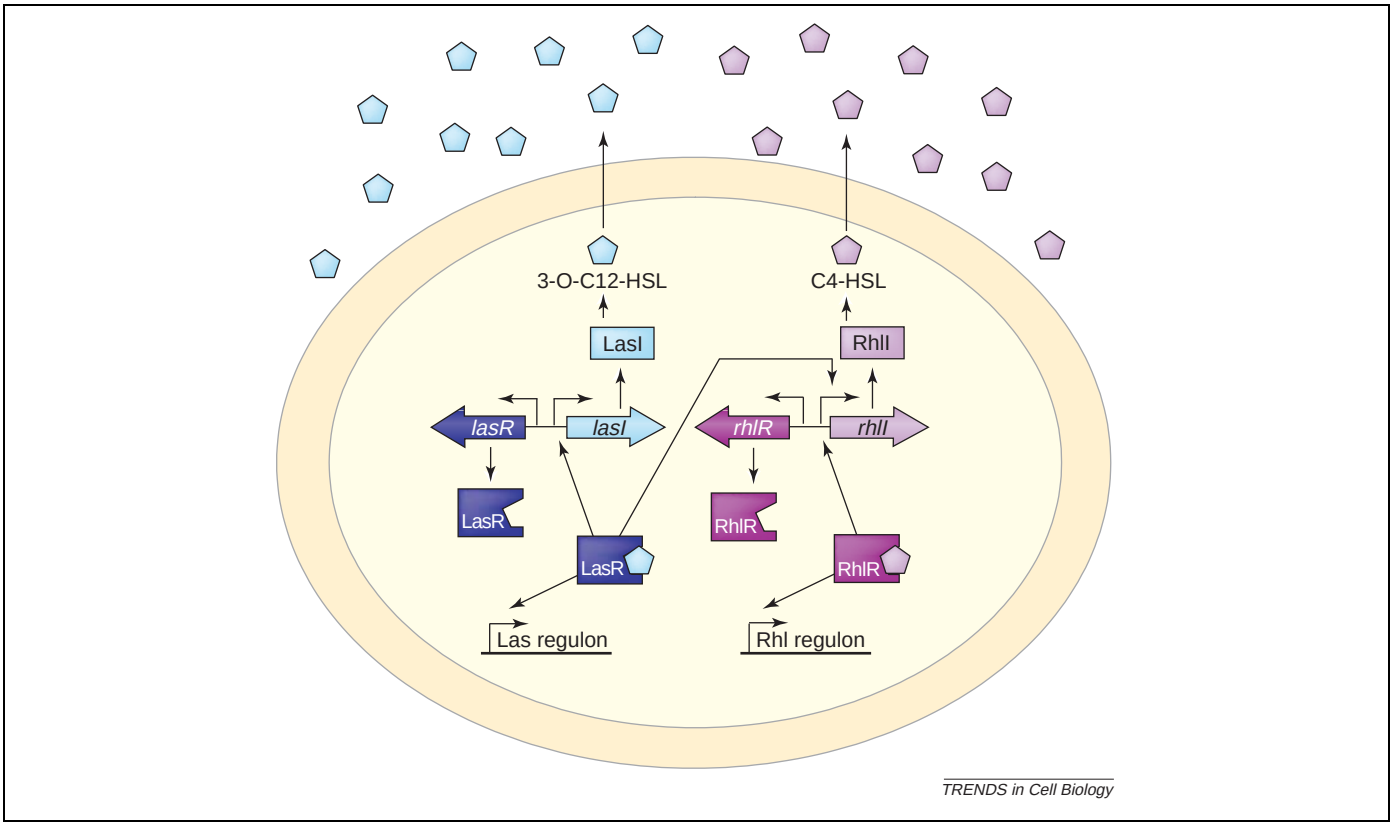

Figure 5. The *Pseudomonas aeruginosa* quorum-sensing system. *P. aeruginosa* uses two LuxI/R-type quorum-sensing systems: LasI/R (blue) and RhlI/R (purple). LasI produces the autoinducer 3-O-C12-HSL (homoserine lactone; blue pentagons), and RhlI makes the autoinducer C4-HSL (purple pentagons). LasR and RhlR bind to their cognate autoinducers and induce gene expression. The systems control partially overlapping sets of genes. Among the genes activated by LasI/R is *rhlI*. Thus, the two quorum-sensing systems function sequentially, and LasI/R-controlled genes are induced before RhlI/R-activated genes. This is a highly simplified figure because many additional regulators and environmental inputs are known to be involved.

the message will accumulate. The use of sRNAs to accomplish an ultrasensitive response could be particularly suitable for 'all-or-none' behaviors such as quorum sensing [40].

Interspecies cell–cell communication

It is becoming increasingly clear that at least some autoinducer signals can be used for interspecies interactions. As mentioned, this notion originated with the finding of the widespread distribution of LuxS and AI-2 and the involvement of the latter in the control of gene regulation in extremely different bacteria [18,41]. Results supporting interspecies communication include the finding that *V. harveyi* detects and responds to cell-free culture fluids containing AI-2 prepared from hundreds of Gram-negative and Gram-positive bacteria [17]. More recently, AI-2 has been shown to be required for mixed species biofilm formation between *Streptococcus gordonii* and *Porphyromonas gingivalis*, members of biofilm communities in dental plaque. Specifically, *P. gingivalis* does not produce a biofilm on glass coated with *S. gordonii* if both species are null for *luxS*. However, introduction of *luxS* into either species promotes the mixed biofilm suggesting that detection and response to AI-2 made by the other species is necessary for development of the consortium [42].

In a surprising finding, *P. aeruginosa*, which does not possess the *luxS* gene and thus does not produce AI-2, responds to AI-2 produced by indigenous (nonpathogenic) host microflora in cystic fibrosis (CF) sputum samples. This result suggests that 'eavesdropping' could be crucial in the CF lung, in which *P. aeruginosa* exists in a complex microbial community composed of pathogens and nonvirulent bacteria. Consistent with this finding, sputum collected from CF patients contains high levels of AI-2, and this AI-2 induces production of *P. aeruginosa* virulence factors such as elastase, exoenzyme T, rhamnolipid and phenazine [43]. Bacterial 'eavesdropping' is not exclusive to AI-2 detection, because *Salmonella enterica* apparently intercepts AHL signals. *S. enterica* has a LuxR-type AHL detector (SdiA) but no LuxI enzyme that could produce a cognate signal. In response to AHLs produced by LuxI-containing Gram-negative bacteria, *S. enterica* expresses the *rck* operon and several other genes that protect *S. enterica* from host defenses in the intestine [44].

Communication between prokaryotes and eukaryotes

Chemical communication extends to the eukaryotic hosts with which bacteria engage in pathogenic and symbiotic relationships. For example, *P. aeruginosa* AHLs enter and are active in eukaryotic cells, in which they alter the immune response [45]. Once inside the host cell, 3-O-C12-HSL stimulates production of the chemokine interleukin 8 (IL-8), which in turn induces the NF-κB transcription factor. 3-O-C12-HSL also controls the production of the Cox-2 enzyme and the signal prostaglandin $(PG)E_2$ [46]. These responses cause recruitment of neutrophils to the lung, in which they contribute to pulmonary inflammation and tissue deterioration. Thus, the *P. aeruginosa* AHLs function concurrently to upregulate bacterial quorum-sensing behaviors and to exploit host immune responses during infection.

The plant pathogen *Agrobacterium tumefaciens* uses AHL-based signaling to control bacteria-to-bacteria exchange of a plasmid containing genes required for virulence [47]. During plant infection, the bacteria deliver this plasmid to the plant, and as a consequence of expression of plasmid-encoded genes, the plant forms tumors. The tumors produce opine molecules, which are used by the bacteria for nourishment. Additionally, opines activate expression of *traR* (a *luxR* homolog) thereby further increasing bacterial–bacterial gene exchange, and increasing the infectivity of the population [48].

Xanthomonas campestris, another plant pathogen, produces a novel α,β unsaturated fatty acid signaling molecule, *cis*-11-methyl-2-dodecenoic acid, which regulates polysaccharide and extracellular enzyme production, both virulence factors. This molecule, called diffusible signaling factor (DSF), is structurally related to farnesoic acid (FA). FA is a signal produced by the fungus *Candida albicans* that inhibits filamentous growth [49]. *X. campestris* DSF also inhibits *C. albicans* filamentous growth and, likewise, at elevated concentrations, FA can regulate *X. campestris* pathogenicity [50]. The molecular mechanism underlying this cross-domain signaling awaits further investigation and the biological significance is not yet known.

Proteomic studies of the legume *Medicago truncatula* reveal that it controls over 150 proteins in response to AHLs produced by *Sinorhizobium meliloti* and *P. aeruginosa*. AHLs also affect the secretion profile of plant compounds that inhibit AI-2 and stimulate AHL quorum-sensing reporter strains. This activity could enable the plant to encourage signaling between AHL-producing bacteria but not AI-2 producers. However, experiments are needed to test the effect of these plant compounds on the bacteria with which *M. truncatula* naturally associates [51].

In a nonantagonistic bacterial–host interaction, *Vibrio anguillarum* AHLs function as chemoattractants for the green alga *Enteromorpha*, which produces motile zoospores that adhere to marine biofilms. There is reduced attachment to biofilms containing *V. anguillarum* autoinducer mutants, and exogenous addition of AHLs or production of AHLs by recombinant *E. coli* restores zoospore attachment. Thus, the bacterial signals benefit both the bacterial and the eukaryotic communities [52].

Finally, recent data suggest that some bacterial and eukaryotic signaling mechanisms are closely related. The bacterium *Providencia stuartii* possesses a protein, AarA, that is related to *Drosophila melanogaster* Rhomboid (RHO) protein. RHO is a serine protease responsible for the intramembrane cleavage, release and activation of epidermal growth factor receptor (EGFR) ligands [53]. These diffusible signals are required for numerous processes including wing vein development. Similarly, AarA releases the *P. stuartii* quorum-sensing signal (hypothesized to be a small peptide) through cleavage of a membrane-bound protein [54]. Remarkably, the *D. melanogaster* and *P. stuartii* RHO and AarA are functionally interchangeable, because introduction of *P. stuartii aarA* into a *D. melanogaster rho* mutant complements the wing development defect, and

D. melanogaster rho introduced into a *P. stuartii aarA* mutant restores quorum sensing [55]. These data suggest that intramembrane proteolysis, signal release, and activation mechanisms are conserved in prokaryotes and eukaryotes and that the released ligands have conserved communication roles in both domains. Interestingly, RHO homologs exist in archaea, bacteria, fungi, plants and humans, suggesting an early and shared evolution of this communication mechanism [53].

Concluding remarks and future directions

Quorum sensing enables prokaryotes to determine their own population density and, in some cases, by detecting interspecies communication signals, bacteria can assess total bacterial numbers in a given environment. Global community behavior can be regulated according to the number of bacteria and species composition of the community, and this regulation could promote survival of the consortia. Although originally thought to be characteristic of higher organisms, the production and exchange of complex mixtures of chemical signaling molecules increasingly seem to be widespread among prokaryotes. Additionally, eukaryotes that exist in either beneficial or hostile associations with quorum-sensing bacteria are being shown to detect and react to the bacterial signals. The future of quorum-sensing research lies in the discovery of additional classes of signals and their molecular mechanisms for regulation of bacterial and host gene expression. Studies so far predict that chemical communication is complicated and involves many signals, often with only subtle variations. A deeper understanding of the complexity of the chemical code and the integrated response to multiple cues will require combined genetic, biochemical, structural, chemical and theoretical analyses. Finally, novel antimicrobial therapies could be developed based on information garnered from quorum-sensing studies, suggesting that research of quorum sensing could have enormous practical impact.

Acknowledgements

This work was supported by NIH grants R01 GM 065859 and R01 AI 054442, NSF grant MCB-0343821, and ONR grant N00014–03–1-0183. We thank the members of the Bassler laboratory and Ned Wingreen for critical reading of the manuscript. We apologize to those authors whose work was not cited owing to space restrictions.

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